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No. 1

THE DESIGN OF THE BALLISTOCARDIOGRAPH

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The purpose of the ballistocardiograph is to measure the movements of the body resulting from the heart beat. These measurements were first studied by Gordon (1) and later by Henderson (2) who suggested that the magnitude of movement is proportional to the volume of blood ejected from the heart per beat. Several investigators have subsequently proposed various designs for ballistocardiographs, and an excellent review of their work has been given by Starr (3).

Before applying the technique to experimental work on animals, it has been considered necessary to make a careful study of the physical requirements of the instrument in order to specify its best possible design; and for convenience in this investigation, human subjects have been used exclusively. As a result of this work, it is possible to evaluate certain factors contributing to the form of the records and to define certain conditions which must be satisfied by the apparatus if a reasonably true record of body movement is to be obtained.

The measurement of the movements of the body which occur as a result of the circulation of the blood is essentially the main problem. Ideally, one could postulate a person floating in free space and with the absence of respiration. The law of conservation of momentum requires that, over the period of one cardiac cycle, the body will acquire no net momentum as a result of the heart beat. If the measurements of the momentary movements of the body could be made under such circumstances, they would represent the *true* ballistocardiogram. In practice, of course, it is necessary to have the subject supported, usually on a swinging bed, and some force must be applied to the supports in order to keep the mean position of the body fixed. The addition of the mass of the support and constraining forces alters the true ballistocardiogram; and, as the constraints on the system are increased, the magnitude of the excursion of the bed is progressively decreased, thereby drastically altering the shape of the record of the movement. Since these constraints provide no additional information, they are justified only if measurements cannot be made without them.

The closest approach to the ideal case was that of Henderson (2) who suspended a very light bed by wires in such a way that it could move only in the longitudinal

direction. The movements were amplified by a lever system and recorded on a kymograph. Henderson's system had a very long period and during a recording the subject was required to suspend respiration. The more recent systems have been highly constrained and do not require cessation of respiration; this apparently makes the measurement simpler but actually introduces errors of unknown magnitude.

THEORY. There are essentially three forces acting in the simplest ballistic system. The first is the force F which it is desired to measure; the second is the elastic restoring force which brings the system back to its resting position once it is displaced, and whose magnitude is proportional to the displacement and opposite in sign; and the third is the damping force which always opposes the motion and in the simplest case is proportional to the velocity. From Newton's second law we may write:

$$m \frac{d^2 x}{dt^2} + b \frac{dx}{dt} + kx = C \cos 2\pi ft \quad (1)$$

where the expression on the right is a single member of the Fourier series representing the forces impressed on the system.

The general solution of this equation is given by Page (4), and, in the ideal case of a system without constraints, reduces to

$$x = \frac{C}{4\pi^2 m f^2} \cos 2\pi ft.$$

The amplitude $a \equiv C/4\pi^2 m f^2$ is a constant for any subject and is the quantity which we are striving to measure with the ballistocardiograph. Note that the appearance of the frequency of the component of the impressed force in the denominator indicates a selectivity for the lower frequency components in the circulatory impacts. This is, of course, inherently the situation in the condition we have defined as ideal.

In going from this ideal case just discussed to more practical cases there are two conditions of interest: 1, the underdamped case in which the damping is insufficient to prevent oscillation and which will exist when $b < 2\sqrt{mk}$; and 2, the critically damped case in which the damping is just sufficient to prevent oscillation and which occurs when $b = 2\sqrt{mk}$.

For the underdamped case the solution of equation (1) is:

$$x = \frac{C}{4\pi^2 m (f_0^2 - f^2)} \cos 2\pi ft$$

The quantity $f_0 = \frac{1}{2\pi} \sqrt{\frac{k}{m}}$ is the natural undamped frequency of the system. In practice f_0 may be determined by removing the damping and counting the number of oscillations per second. The underdamped case demonstrates the phenomenon of resonance whenever $f = f_0$, and the amplitudes of these frequencies appear excessively large. Resonance can be practically avoided by making f_0 either very large or very small. The former would be the choice for high frequency recording in a system which remains a simple mechanical system as the constraints on the system are increased.

For the critically damped case the solution of equation (1) is:

$$x = \frac{C}{4\pi^2 m (f_0^2 + f^2)} \cos (2\pi ft - \phi)$$

$$\text{where } \tan \phi = \frac{2ff_0}{f_0^2 - f^2}.$$

This situation avoids resonance completely but again requires f_0 to be either very large or very small in order to minimize the phase shift.

The ratio of the amplitude of this case to the ideal case gives:

$$\frac{a_1}{a} = \frac{f^2}{f_0^2 + f^2} \quad (2)$$

which shows that, for small values of f_0 , the recording will be very close to the ideal with only small errors in amplitude and phase.

Figure 1 shows a series of curves plotted from equation (2). The ordinates represent a_1/a , the abscissa gives the natural undamped frequency of the bed, and the several curves are for a range of impressed frequencies to be expected in a Fourier analysis of the normal human ballistocardiogram. By examination of these curves it is possible to estimate for any bed frequency the errors introduced into the various frequency components of the ballistocardiogram. It is obvious then that in order to record the movements of the body, as if it were floating freely in space, the system should be of low frequency and critically damped.

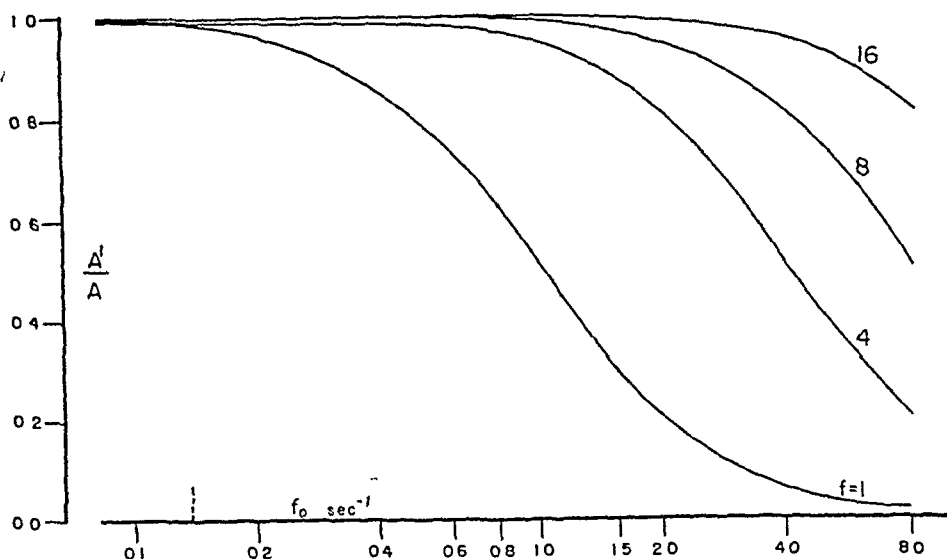


Fig. 1. The abscissa is the undamped bed frequency; the ordinate, the ratio of the amplitude of the observed response to the amplitude of the ideal response for the bed under critical damping. The curves represent the amplitude ratio for several impressed frequencies (f).

Because of the ease of recording a high frequency bed is often used. This procedure assumes that the subject and bed remain a simple mechanical system in which the subject can be represented ballistically by a mass equal to his weight. However, the increased restoring force applied to the bed to provide its high frequency response accentuates the actual situation, i.e., that the subject and bed are two elastically coupled systems each having elasticity and damping. In the construction of a bed it is necessary, therefore, that the constraining forces be kept to a practical minimum, otherwise the movements of the bed will be determined by the elastic properties of the tissues as well as by the cardiac output. It is evident that a critically damped bed with a very low natural frequency should be used, and departure from this condition will introduce definite errors.

APPARATUS. The bed, which consists of a light wooden board covered on top by a thin woolen blanket and has a head-rest at one end, is shown in figure 2. The board was placed on a wooden framework supported by four long, heavy steel

springs, *S*, 2 inches wide and $\frac{3}{32}$ inch thick which are fastened by means of iron clamps, *B*, to the four legs of a heavy wooden table. By adjusting the position of the clamps the spring length may be varied quickly and easily over a wide range. This arrangement made it possible to obtain a very low frequency bed, yet one which is rugged, portable and without friction. Very low frequencies are possible because the length of the springs can be increased until the restoring force which they introduce is just sufficient to support the weight of the body. Provided the positive restoring force introduced by the springs is just greater than the displacing force due to the essentially instable position of the body, it will be a long period system. The total weight of the moving part of the bed is about thirty-five pounds. The permitted direction of movement of the bed is only longitudinal.

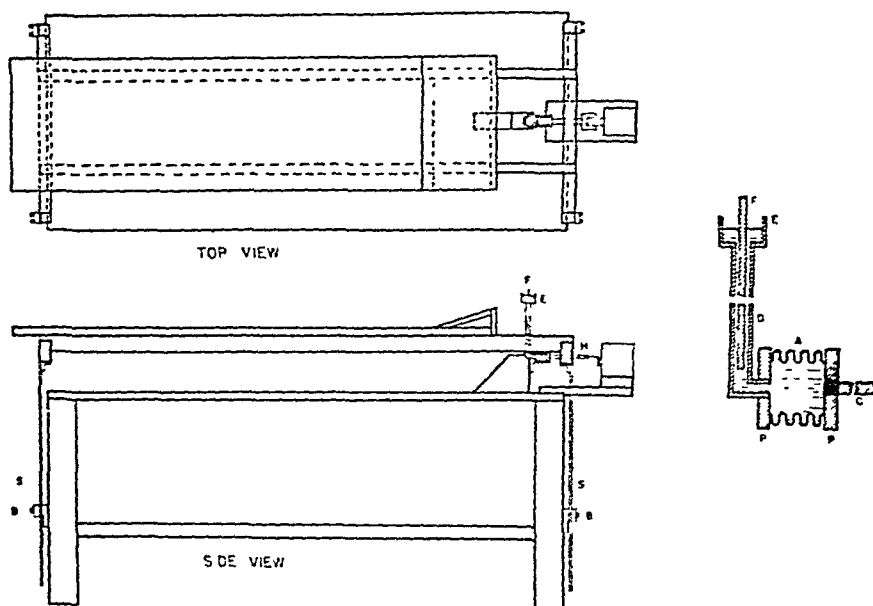


Fig. 2. Illustrates two views of the bed and a detailed drawing of damping device.

The construction of a satisfactory damping system presented some difficulty. The requirement that it be completely free from friction, yet capable of providing a large continuously adjustable amount of damping, precluded the use of all customary devices. Mechanical dampers were found to introduce friction; and magnetic dampers and vanes moving in oil provided an insufficient amount of damping. Figure 2 includes an illustration of the light metal sylphon bellows damper which we found to be completely satisfactory. This bellows, *A*, (which has a restoring force of 0.5 lb. for a deflection of 0.100") is soldered between two thick brass plates, *P*, one being attached to the bed by a brass rod, *C*, and the other clamped to the table. The brass tubing, *D*, leads from the inside of the bellows to the reservoir, *E*, and a brass rod, *F*, is selected to fit snugly inside tube *D*. The bellows, tube, and reservoir are filled with a light oil, so that movements of the bed cause the oil to flow through the tube *D*, thus providing the damping resistance to the bed movements. By varying the size and depth of the

plunger rod, F , any desired amount of damping can be obtained. The elastic restoring force introduced by the bellows merely adds to that provided by the springs supporting the bed and becomes an appreciable factor only when extremely long period beds are used. In filling the bellows system with oil it is imperative that air bubbles be excluded. The presence of these bubbles can be detected by displacing the bed and observing the recovery pattern. Perfect recovery curves are shown in figure 3, D , E and F . The presence of air bubbles will superimpose a high frequency oscillation on the pattern.

It was found desirable to have a frictionless recorder which would give an immediate record without the necessity of photographic development. To accomplish this an air condenser (fig. 2, H) was constructed, one plate of which was stationary and the other moved with the bed in such a way that the movements of the bed gave a linear change of capacity. The condenser was incorporated in a high frequency vacuum tube oscillator so that capacity changes produced linear changes in oscillator plate current (5). These current changes were

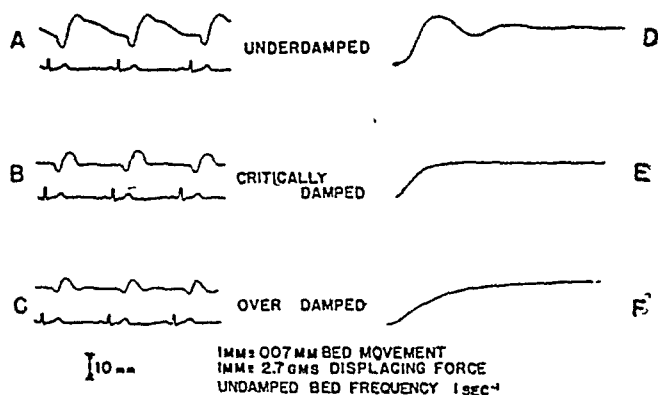


Fig. 3. Records A , B and C are from an 80 kgm. subject under three conditions of damping; D , E and F demonstrate the corresponding damping record with 80 kgm. of dead weight.

amplified by a three-stage, direct-coupled amplifier and used to activate an ink-writing oscillograph. It was necessary to exercise considerable care to insure linearity between the motion of the bed and the movements of the pen. A second pen on the ink-writer records the electrocardiogram simultaneously with the ballistocardiogram. A paper speed of 25 mm. per second was found satisfactory. This recording system has been found to be excellent as a research method, but is not necessarily recommended as a clinical instrument.

There are several general precautions which the authors consider important. The bed and table must be of sturdy construction; the table should be made of heavy materials and the bed of light materials (the lightness and rigidity of the bed being attained by wooden construction). To reduce the effect of secondary oscillation, the subject should be as near as possible to the center of the bed, the damper applied exactly on the long axis of the bed, and, most important, the recording device should be attached directly to the moving part of the damper.

RESULTS. Extensive series of records were made on four normal healthy subjects. These series consisted of measurements made over a wide range of bed

frequencies, and for each frequency several different damping conditions were used. In cases where basic concepts were being verified more subjects were tested, and in one instance records were obtained on 23 subjects of both sexes ranging in age from 20 to 72 years and in weight from 105 to 195 pounds. In preparation for each record a weight equal in mass to that of the subject was placed on the bed. The plunger was removed from the damper and a record of the undamped natural frequency obtained. By means of a micrometer screw, the bed was displaced a measured amount and the corresponding deflection of the writing pen recorded: a known force was then applied to the bed and the deflection of the pen recorded. The damping plunger was then replaced, the bed slightly displaced, and its return to rest recorded. From the record the state of

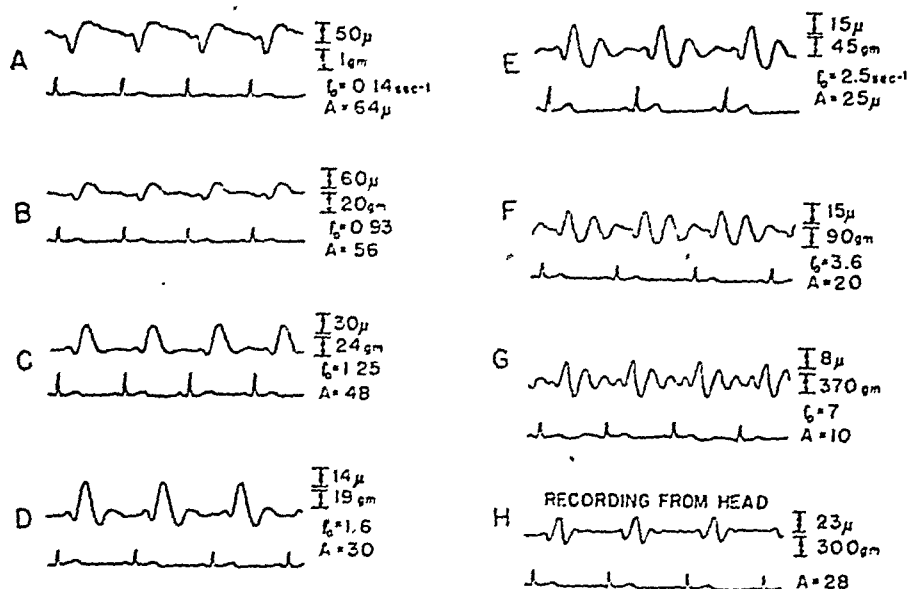


Fig. 4. A, B, C, D, E and F are a series of records on a 72 kgm. subject with the bed critically damped. With each record is shown the deflection corresponding to the linear movement of the bed in microns; the deflection for a static displacing force in grams; the frequency of the bed when undamped, and the amplitude of the oscillation. Record G is from the same subject for a higher frequency undamped bed; record H, the same subject lying on the floor with the pick-up device attached to his head.

damping could be judged and the plunger moved in or out until the desired degree of damping was attained. Three such records are shown in figure 3 (D, E and F).

The subject ascended the bed and rested for a few minutes before the record was taken. The elimination of respiration was necessary only on the lower frequency bed, but for uniformity all the records were taken with the respiration arrested in midposition. An instructive series of records is demonstrated in figure 4 (A to F). These were taken on a single subject, all with critical damping over a range of frequencies from 0.14 to 3.6 per second. Since the amplification was changed considerably from record to record, both displacement and force calibrations are included. The amplitude A is given in order to enable the reader to obtain a clearer understanding of the magnitude of the bed displace-

ment. The amplitude is defined as the sum of the two principal waves of the ballistocardiogram (waves *I* and *J*, Starr et al. (3)). The type of pattern obtained on a high frequency undamped bed is shown in figure 4, *G*. This type of bed is similar to that used by Starr (3) and the results are comparable. Records taken on other normal individuals under the same conditions vary in only minor details.

The fact that the form and size of the ballistic record is almost completely a function of the way in which it is taken, and varies only in minor respects between normal individuals, is regarded by the authors as the most striking conclusion to be drawn from this research.

The series of records in figure 4 demonstrates the relatively large change in amplitude and form occurring with change in bed frequency. Record *A* ($f_0 = 0.14 \text{ sec}^{-1}$) represents a very close approximation to the ideal. This is indicated by two facts: 1, the fundamental frequency of the ballistocardiogram ($f \doteq 1 \text{ sec}^{-1}$) is well above the bed frequency so that the expected errors are small. Thus, in figure 1 the dotted line at $f_0 = 0.14 \text{ sec}^{-1}$ shows the maximum error for $f = 1 \text{ sec}^{-1}$ as 2 per cent and for higher frequency components the error is even less; 2, the necessity of increasing f_0 about seven-fold before any considerable change in shape or size of the record is noted suggests that no appreciable change in the record would have been observed if f_0 had been made infinitesimally small. As f_0 is further increased by relatively small amounts, large changes in size and shape of the pattern appear. Examination of figure 1 shows that these large changes could be due to a considerable contribution from the second harmonic, a frequency about twice the heart rate. The effect of increasing the bed frequency (record *B*) has been a decrease of the amplitude, *A*, and a return of the record to the base line sooner than when the restoring force was smaller (record *A*). This is in striking agreement with the theory we have outlined above. In record *C* the curve not only returns to the base line sooner but actually goes slightly below the line. The effect is accentuated in records *D* and *E*, and at the highest frequency *F*, there is considerable oscillation before the curve returns to the base line. Since this whole series involves critical damping, the oscillatory motion cannot be explained on the basis of the simple theory. A logical assumption is that these oscillations are caused by the elastic properties of the tissues of the subject, the effects becoming more important at bed frequencies above 1.5 per second. Since most of the recent work by other investigators has been done with high frequency beds, it was necessary to test this assumption.

Tissue elasticity was discussed by Starr (3) who obtained records with and without footboard and stoppers on the subject. He concluded that there was no relative motion between the subject and the bed, but recognizing the importance of tissue elasticity he attempted an elucidation by tapping warm cadavers on the head and comparing the patterns produced with those from the tapping of an equal dead weight. He found that beds containing dead weight produced a frequency of 10 per second and gave, with the cadavers, frequencies of 5 to 6 per second. Since the variation was within 20 per cent among a varied group of bodies, he concluded that the tissue elasticity was essentially constant. We do

not, however, consider that these conclusions are proven and have demonstrated the importance of tissue elasticity in the following ways.

1. Rubber tubes were placed between the board on which the subject lay and the supporting frame of the bed. The result (fig. 5) was an accentuation of the oscillatory effect.

2. Records on stout subjects should show more overshoot and oscillation than records made on thin subjects of the same weights (fig. 6). Both subjects had substantially the same form of record at low frequency.

3. The important rôle played by tissue elasticity is clearly shown in figure 4, *H*. The subject lay upon the floor with the recording device attached to his head. The amplitude of the oscillation of the subject on his own tissues is about 45 per cent of the amplitude of the ideal (fig. 4, *A*), and nearly three times as large as the record taken from a high frequency undamped bed (fig. 4, *G*).

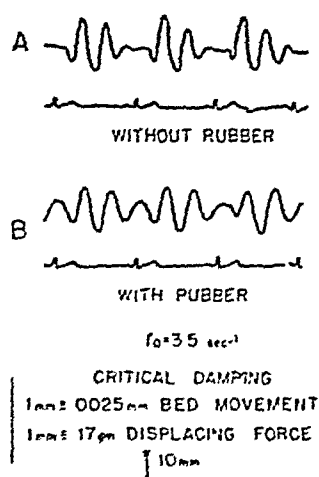


Fig. 5

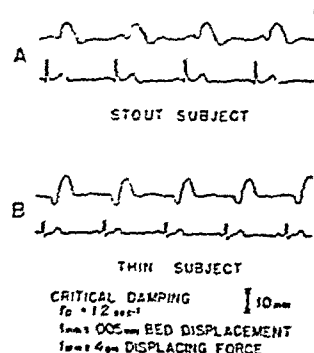


Fig. 6

Fig. 5. Record *A* illustrates a 72 kgm. subject on a moderately high frequency critically damped bed. Record *B* demonstrates the changes introduced by placing rubber tubing between the board on which the subject lies and the frame of the bed, the ballistic movements being thus transmitted from the subject to the recorder through a layer of rubber.

Fig. 6. Records on two 80 kgm. subjects of different body shapes.

It appears, therefore, that with high frequency beds the elastic properties of the tissues determine almost completely the size and shape of the record. It is necessary then to work with critically damped beds of which the frequencies, when undamped, are less than 1.5 per second. The selection of a low frequency bed permits the body to be considered as dead weight and allows the observer to specify rigidly the conditions of restoring force which will enable one to disregard errors introduced by tissue elasticity.

Another view of the origin of these oscillations is presented by Hamilton and Dow (6, 7) who suggest that they are caused by impacts of the blood at peripheral boundaries and by surgings of the blood within the aortic windkessel. The momentum changes involved in these impacts are of considerably smaller magnitude than the initial impulses due to the footward and headward recoils at the time of the ventricular systole, and the subsequent headward impulse produced

by the diastolic run-off around the aortic arch. For this reason we feel that high frequency undamped recording overaccentuates, by resonance and by other factors, the high frequency components of the cardiac cycle to the detriment of the lower frequency events more closely related to the cardiac stroke.

So far we have considered solely the case of the critically damped bed. From theoretical considerations it is not necessary to consider either the overdamped or the underdamped situation. However, numerous records were taken under both conditions in order that no practical point be overlooked. Figure 3 shows three records taken on the same subject on a bed with a natural frequency of 0.90 per second. The effects of respiration were more marked with underdamping than with critical damping. With overdamping, although the respiratory movements are reduced, the overshoot due to tissue elasticity enters at lower bed frequencies than with critical damping. It is concluded that overdamping provides no significant advantage which could not be obtained by using a higher frequency bed and applying critical damping to it.

Several records were made with the subject lying at various angles to the direction of the motion of the bed and, whereas the motion was by no means zero when

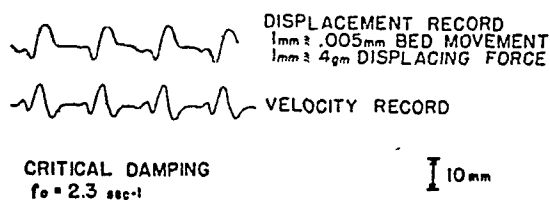


Fig. 7. Comparison of a displacement record and a velocity record taken simultaneously on a 72 kgm. subject.

the body was at right angles to the direction of movement of the bed, still no angle was found which would give a greater deflection than was given by the body lying parallel to the direction of recording. It was found also that it made little difference whether the subject lay on side, back or stomach.

The applicability of the ballistocardiograph patterns to the measurement of the cardiac stroke volume arises from a consideration of the law of conservation of momentum (*i. e.*, the change in momentum of the blood must equal the recoil momentum of the body). According to Henderson's (2) interpretation the major deflection of the ballistic record is caused by the momentum change of the blood as it passes around the arch of the aorta. Since the momentum is the product of mass and velocity it follows that, if the form of the pulse wave is unvarying, the mass of blood moved (or the cardiac stroke volume) should be related to the velocity of the bed as well as to the ballistic throw or displacement. In this investigation we have recorded the displacement in all cases, and in several instances the velocity was recorded. The latter record was obtained in several ways: 1, by connecting a long solenoid to the bed and attaching a fixed bar magnet extending part way into the coil; the current generated, which was proportional to the velocity of the bed, was then amplified and recorded; 2, by placing small coupling condensers between the stages of the amplifier used in the displacement pickup, it was found possible to differentiate electrically the displacement curve and thereby obtain the velocity record. Records taken by the second method

are shown in figure 7 which gives both displacement and velocity curves recorded simultaneously. The choice between displacement and velocity recording is now undergoing experimental tests, and we expect to present in a subsequent paper an empirical equation relating the cardiac stroke volume and the dimensions of the recorded pattern.

DISCUSSION. It can be stated definitely that the bed should be critically damped by some device which offers no friction in the conventional sense. The contention that external damping is not necessary because the body provides all the damping that is required (3) is apparently only partly true and leads to serious error. Inspection of figure 4 shows that a high frequency undamped bed will measure only 15 per cent of the total movement of the ideal case. This 15 per cent is dependent on the type of tissue of the subject, the amount of fat, the degree of hydration, the tonus of the muscles, etc. In our experience subjects measured on a low frequency, critically damped bed provide patterns which are quite constant from day to day.

It has been found that the natural frequency without damping should be as low as possible, the only limiting factor being convenience. Measurements at frequencies less than 1 per second have been found difficult, but by no means impossible to make. Respiration must be suspended, and the subject must remain absolutely motionless during the recording. Such records are almost true graphs of the motion of the body as a result of the action of the heart. At frequencies of one per second the amplitude of the wave is reduced only about 12 per cent and the wave form is distorted little. Respiration must be suspended, but otherwise the recordings are not difficult to make. If measurements are performed on cooperative subjects, this would be the frequency of choice. At frequencies of 1.5 per second the amplitude is reduced to about 55 per cent of the value determined by the low frequency bed; the wave form, though considerably altered, may be sufficiently accurate for some purposes. Quiet breathing is permissible, and the records are quite easy to obtain. It is not recommended that frequencies higher than 1.5 per second be used, since errors become excessive above this point. These increased errors are caused by the rise in bed frequency (fig. 1), *i. e.*, by the imposition of greater constraints which prevent the bed and body from moving in unison. Complex patterns of movement are thereby produced; these bear little resemblance in size or shape to the actual motion. These higher frequencies are tempting to use since the records are very easily obtainable.

The measurements of Starr et al. (3) were made on a high frequency, undamped bed, and it must be supposed therefore that the formula developed by these authors was largely empirical. The cardiac output as computed by their formula gave reasonably good agreement with measurements obtained by independent methods, and this work has been substantiated independently by Cournand et al. (8). In view of the evidence presented here this agreement is quite surprising, and it must be presumed that the empirical approach takes account of the subject's elastic properties by the introduction of body factors (*i. e.*, the surface area).

One of the most serious objections to the clinical use of the ballistocardiograph has been the overlapping of patterns occurring with high heart rates. In the

past these measurements have been made with high frequency undamped beds, with which, even for the slow normal heart, the oscillation due to one heart beat would barely die out before the next beat commenced. However the use of a low frequency critically damped bed demonstrates (fig. 4, C) that the major portion of the pattern occupies an interval approximately equal to the time between the start of the Q-wave and the end of the T-wave. It may be concluded therefore that, if the record is properly taken, it is possible to increase the heart rate by a factor of two or more without expecting serious errors. Records taken on normal subjects immediately after exercise have substantiated this conclusion.

SUMMARY

An experimental and theoretical study of the human ballistocardiograph has been undertaken with a view to designing an apparatus which will measure accurately and easily the motion of the body which occurs as a result of the heart beat. Both approaches to the problem have demonstrated that:

a. It is absolutely necessary to have the bed damped by a device which introduces no friction. Since both over and under damping yield errors which are difficult to evaluate, the damping should be critical for the total load.

b. The restoring force on the bed is most easily specified by the undamped frequency of oscillation of the loaded bed. True ballistic records are obtained only when frequencies somewhat less than 1 per second are used. When higher frequencies are used, the errors in amplitude and wave form increase with the frequency.

c. Measurements at very low frequencies are difficult to make. However at frequencies between 1.0 and 1.5 per second, the difficulties are reduced somewhat and the errors are not serious.

d. It was found that at frequencies above 2 per second the subject and the bed do not move in unison, and the pattern recorded depends in part on the elastic properties of the tissues of the subject. This leads to serious errors at high frequencies.

Complete specifications are given for the construction of the bed used in this work.

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REFERENCES

- (1) GORDON, J. W. *J. Anat. and Physiol.* 11: 533, 1877.
- (2) HENDERSON, Y. *This Journal* 14: 287, 1905.
- (3) STARR, I., A. J. RAWSON, H. A. SCHROEDER AND N. R. JOSEPH. *This Journal* 127: 1, 1939.
- (4) PAGE, L. *Introduction to theoretical physics*. D. VanNostrand Co., Inc., New York, p. 66.
- (5) LILLY, J. C. *Rev. Scient. Instruments* 13: 34, 1942.
- (6) DOW, P AND W. F. HAMILTON. *This Journal* 133: 263, 1941.
- (7) HAMILTON, W. F. AND P. DOW. *This Journal* 133: 313, 1941.
- (8) COUNNAND, A., H. A. RANGES AND R. L. RILEY. *J. Clin. Investigation* 21: 287, 1942.

A STUDY OF THE SUBSTANCES IN BLOOD SERUM AND PLATELETS WHICH STIMULATE SMOOTH MUSCLE¹

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It has been known for many years that serum and defibrinated blood stimulate smooth muscle. (For reviews, see Janeway, Richardson and Park, 1918; Werle, 1935; Gaddum, 1936; Amberson, 1937; Rigler, 1938; and Reid and Bick, 1942.) This property of serum is attributed to several substances called "Spätgifte" (Freund, 1920), the chemical constitution of which is unknown (Stewart and T. F. Zucker, 1913; Wu, Fang and Tsai, 1942). One or more of these substances reduces the blood flow of perfused mammalian organs to a low value unless the lungs or some other organ capable of destroying the vasoconstrictor material is included in the perfusion circuit (Eichholtz and Verney, 1924; Bing, 1941). Blood and plasma prevented from clotting by anticoagulants usually fail to stimulate smooth muscle. However, active substances can appear in the absence of clot formation (Trendelenburg, 1915-16; Freund, 1920; Reid and Bick, 1942; Landis, Wood and Guerrant, 1943), since the essential factor in the development of activity in shed blood appears to be the breakdown of the blood platelets (O'Connor, 1912; T. F. Zucker and Stewart, 1913; Janeway et al., 1918; Freund, 1920; and Reid and Bick, 1942).

The work presented in this paper consists of three parts. First, some of the studies of the earlier investigators were confirmed and extended. Secondly, a fraction of the buffy coat of human blood was prepared and its chemical and pharmacological properties investigated. Finally, a study was made of the destruction of one of the smooth muscle stimulating substances by various tissues *in vitro*.

METHODS. The activity of various fractions of blood, the preparation of which will be described below, was tested on smooth muscle obtained from various sources. The contraction of the sensitized (sympathetically denervated) nictitating membrane (n.m.) of the cat (nembutal anesthesia) was recorded by means of an isotonic lever with a magnification of about 10. The test materials in a volume of 2 cc. were injected through the cannulated carotid artery. The artery could be recannulated in three subsequent experiments.

Sections of rat duodenum or of rabbit or guinea-pig ileum were suspended in 10 to 20 cc. of Tyrode solution maintained at 38°C, and their contractions recorded under about 1 gram of tension. Strips of ox carotid artery, prepared by the technique of Janeway et al. (1918), or the uterus of the virgin rat was suspended in 20 cc. of Ringer solution. The recording lever magnified the responses of the indicators about 4½ times.

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The vessels of the cat tail, cat ear, or rabbit ear were perfused with Ringer solution (0.9 per cent NaCl, 0.042 per cent KCl, 0.024 per cent CaCl_2 , 0.03 per cent NaHCO_3 , 0.1 per cent glucose) at a pressure between 30 and 70 cm. H_2O . The rate of outflow, as recorded by a drop counter, was determined for one minute at the height of the constrictor response, which usually began 0.5 to 1 minute after the injection of 0.1 to 1.0 cc. of test material into the tubing above the cannula.

In experiments in which the blood flow through the kidney was measured, the lungs and kidney of a cat were simultaneously perfused by the method of Bing (1941), using defibrinated cat blood at 35°C . The test material was dissolved in a few cubic centimeters of fluid withdrawn from the perfusion circuit. The solution was brought to 35°C and injected into the renal arterial inflow. Renal blood flow, which was not affected by control injections of the perfusate, was determined by measuring the venous outflow.

RESULTS. *The action of shed blood on smooth muscle.* The injection of 0.1 to 1.0 cc. of cat, dog, human or rabbit serum produced a contraction of the n.m. The sera of the cat and rabbit were usually more active than those of the dog and man, but there was considerable individual variation. Ox carotid artery strips showed a pronounced response to 0.25 cc. of rabbit serum or to 1 cc. of cat serum. One cubic centimeter of human serum produced only a slight contraction of this indicator. The sympathetically denervated pupil of the cat responded to the intracarotid injection of 0.3 to 1 cc. of cat serum with a pronounced constriction, which was preceded by a slight dilatation.

In 9 out of 11 instances, 1 cc. of fresh, citrated cat plasma, obtained by centrifuging blood (2700 r.p.m.) to which one-tenth volume of 5 per cent sodium citrate had been added, had no action on the n.m. Plasma which had been refrigerated for 24 hours was usually active. The addition of citrate to serum did not modify its effect on the n.m. One-half cubic centimeter of pooled citrated human plasma taken from bank blood which was 7 to 10 days old caused a pronounced contraction of the n.m. In two experiments the effect of citrated rabbit plasma on the rat intestine and on the vessels of the rabbit ear was studied. The vasoconstriction produced by 0.4 cc. of plasma was approximately one-fourth as great as was the response to an equal volume of serum. The rat intestine failed to respond to 0.2 cc. of plasma, although 0.1 cc. of serum produced a marked contraction.

The origin of the active substances was studied by testing the activity of lysed cat blood platelets, white cells and red cells on the n.m., and of similar fractions of rabbit blood cells on the rat intestine and vessels of the rabbit ear. The cells were prepared from citrated blood (one-fiftieth volume of 20 per cent sodium citrate) which was centrifuged at approximately 1200 r.p.m. The platelet-rich plasma was removed and the sedimented cells were washed three times with a salt-citrate solution (Chargaff et al., 1936). The layer of white cells was drawn off and combined with the sediment obtained from slow centrifugation of the platelet-rich plasma as the white cell fraction. Platelets were obtained by rapid centrifugation of the plasma. The cells were disintegrated in distilled water, made isotonic with double concentrated Ringer solution, and centrifuged. The three fractions were each made up to the volume of blood from which they were

derived (10 cc.), thus making it possible to compare the amount of active substance in the three types of cells and in defibrinated blood or serum. A sample of the platelet fraction caused a greater contraction of all three indicators than did an equal sample of the red cell fraction (tables 1 and 2). This demonstrates that 4 cc. of red cells contain less active material than does about 0.05 cc. of platelets, and hence that the concentration of the active substances in the platelets is at least 80 times that in the red cells. Extracts of red cells which were prepared

TABLE 1

The response of the nictitating membrane of the cat to certain fractions of cat blood

EXPT.	MATERIAL	cc.*	RESPONSE
1	Defibrinated blood.....	0.5	++
	Lysed platelets.....	1.0	+++
	Lysed red cells.....	1.0	0
2	Lysed platelets.....	2.0	+++
	Lysed red cells.....	2.0	++
3	Defibrinated blood.....	1.0	++++†
	Lysed platelets.....	1.0	0
	Lysed red cells.....	1.0	+++
4	Defibrinated blood.....	1.0	+++
	Lysed platelets.....	1.0	+++
	Lysed red cells.....	1.0	0
5	Defibrinated blood.....	1.0	+++
	Lysed platelets.....	1.0	+++
	Lysed red cells.....	1.0	+++
6	Defibrinated blood.....	1.0	++++
	Lysed white cells.....	1.0	++++
	Lysed red cells.....	2.0	0
7	Lysed platelets.....	1.0	+
	Lysed white cells.....	1.0	++
	Lysed red cells.....	1.0	0

* The lysed cell fractions were made up to the volume of blood from which they were prepared (10 cc.) in order to facilitate their comparison.

† An exceptional experiment in which an unusually small volume of platelets was obtained.

from defibrinated blood had less effect on the n.m. than did similar fractions prepared from citrated blood. This indicates that the active substances in this fraction do not originate from erythrocytes, but from platelets or white cells. Platelets are probably responsible for the activity of the white cell fraction, which was approximately equal to that of the platelet fraction. The activity of defibrinated blood as tested on the n.m., and of serum and an extract of the clot as tested on the rabbit ear vessels or rat intestine was not greater than that of the

cell extracts (tables 1 and 2). This observation indicates that, although coagulation releases the active constituent from the platelets, it does not cause the production of any additional smooth muscle stimulating substances.

To determine whether or not the active substance originated from the white cells as well as from the platelets, the activity of lysed lymphocytes was investigated. Lymphocytes were obtained by centrifuging 2 to 11 cc. of heparinized lymph taken from the thoracic ducts of dogs or cats under nembutal anesthesia. In some instances, pressure was exerted on the abdomen to facilitate the collection of the lymph. The lymphocytes were disintegrated in the same manner as were the blood cells, the final volume of the extracts being 2 to 4 cc. The results (table 3) demonstrate that the lymphocytes from 1 to 5 cc. of heparinized lymph (about 0.05 cc.), when lysed, failed to stimulate the n.m. or rat intestine.

TABLE 2

The response of the rat intestine and the blood vessels of the perfused rabbit ear to certain fractions of rabbit blood

TEST MATERIAL	RABBIT EAR		RAT INTESTINE	
	cc.*	Reduction in flow <i>per cent</i>	cc.*	Response
Serum.....	0.2	91		
Extract of cells and clot.....	0.2	62		
Lysed platelets.....	0.2	81		
Lysed white cells.....	0.2	82		
Lysed red cells.....	0.4	45		
Serum.....	0.4	71	0.1	++
Extract of cells and clot.....	0.4	48		
Lysed platelets.....	0.4	62	0.2	++++
Lysed white cells.....	0.4	69	0.2	++++
Lysed red cells.....	0.4	12	0.2	Inhib.

* All of the fractions were made up to the volume of blood from which they were prepared (10 cc.) in order to facilitate their comparison.

Since the extract of an equal volume of platelets has a pronounced effect, these findings demonstrate that lymphocytes contain at most a low concentration of the active substances. One to 2 cc. of clotted or heparinized lymph caused a decrease in the tone and amplitude of contraction of the gut. The action of 0.05 cc. of serum was only slightly decreased by mixing it with 2 cc. of lymph, which suggests that the inhibitory material did not mask the presence of a stimulating substance. Lymph produced a constriction of the blood vessels of the perfused rabbit ear and usually elicited a contraction of the n.m.

Some properties of the smooth muscle stimulating substances in shed blood. Wu et al. (1942) found that the fraction of rabbit serum which dialysed through parchment membranes stimulated the rabbit intestine but failed to constrict the blood vessels of the rabbit ear, whereas the dialysed serum had a vasoconstrictor action. This experiment indicates the existence of at least two smooth

muscle stimulating substances in rabbit serum. The results obtained in the present investigation demonstrate that the two substances cannot be separated by dialysing serum through cellophane membranes (Visking sausage casing), since the constituent of rabbit serum which stimulates the vessels of the rabbit ear, as well as the material in both cat and dog serum which contracts the n.m. and rat intestine, passes through these membranes.

TABLE 3

The response of the rat intestine, cat nictitating membrane and the blood vessels of the perfused rabbit ear to lysed lymphocytes and to lymph

EXPT.	MATERIAL	RAT GUT		N.M.		RABBIT EAR	
		cc.	response	cc.	response	cc.	response
1	Clotted dog lymph*.....	1.0	Inhib.				
	Lysed dog lymphocytes.....	1.0†	0				
2	Heparinized dog lymph.....	1.0	Inhib.	2.0	+++	1.0	++++
	Lysed dog lymphocytes.....	1.25	0	5.0	0		
	Rabbit serum.....					0.1	++
	Cat serum.....	0.05	+++	0.3	++++		
	Cat serum in 1 cc. lymph.....	0.05	+ or ++				
3	Heparinized dog lymph*.....	2.0	Inhib.	2.0	0		
	Lysed dog lymphocytes.....	2.5	0	5.0	0		
	Rabbit serum.....	0.05	+++	0.3	++++		
	Rabbit serum in 1 cc. lymph.....	0.05	++				
4	Heparinized cat lymph.....	1.0	Inhib.	2.0	++++		
	Lysed cat lymphocytes.....			5.5	0		
	Cat serum.....			0.4	+++		
5	Heparinized cat lymph.....			1.0	+++	0.5	+++
	Clotted cat lymph.....			1.0	+++		
	Heparinized cat lymph (from expt. 4, 1 day old).....			1.0	++++	0.5	+++
	Lysed cat lymphocytes.....			3.1	0		
	Cat serum.....			0.3	++++	0.1	++

* No pressure was exerted on the abdomen during collection of the lymph.

† The dosage of lysed lymphocytes represents the volume of lymph from which the lymphocytes were obtained.

Experiments were performed which indicate that the material in serum which contracts the n.m. is not histamine, tyramine or a choline ester. It was found that the activity of cat serum as tested on the n.m. was not diminished by incubation of the serum with histaminase. The response of the unsensitized n.m. to serum was increased by the intravenous injection of cocaine HCl (8 mgm./kgm.). In agreement with the results of Daly et al. (1937), the response of the n.m. to 1 mgm. of tyramine was found to be decreased by cocaine. The intravenous injection of atropine (1 mgm./kgm.) failed to affect the response of the n.m. to serum, but abolished the n.m. response to mecholyl.

In two experiments it was found that, although acetone extracts of serum, made in the same manner as were the extracts of incubated serum, were less active than whole serum on the n.m., rat intestine and the tail vessels of the cat, the extracts possessed the same fraction of the activity of serum when they were tested on any one of the three indicators. This suggests that the substances which stimulate the three types of smooth muscle are chemically similar, and may in fact be identical.

Some properties of an active fraction prepared from the buffy coat of human blood. A concentrated preparation of the smooth muscle contracting material (subsequently referred to as SMC) was made from the buffy coat² of human citrated blood which was approximately 24 hours old. After centrifugation and removal of the plasma from 50 liters of blood, the buffy coat, mixed with about 2.5 liters of red cells and plasma, was drawn off. A second centrifugation yielded 500 cc. of buffy coat. Cell counts made on one sample showed that 14,640,000 platelets, 480,000 white cells and 240,000 red cells/cu. mm. were present. Smears demonstrated the presence of many lymphocytes but of only a few degenerating neutrophils. The buffy coat was added to 500 cc. of acetone. After standing in the cold overnight, the mixture was filtered, and the precipitate washed several times with 60 per cent acetone. The acetone was evaporated from the water-clear, yellow filtrate and washings, which were then acidified to approximately pH 6.0 with HCl, chilled, and extracted three times with cold ether. The aqueous layer was neutralized and evaporated to dryness in vacuo at 65°C. The yellow solid was extracted with absolute methanol. One-half to 2 grams of insoluble whitish material of low activity were discarded. When the methanol solution was dried, about 1.5 grams of brownish-yellow, transparent, hygroscopic material (SMC) was obtained. Since tests on the blood pressure of the atropinized cat indicated that SMC contained approximately 0.5 gamma of histamine per mgm., SMC was dissolved in water, autoclaved for 10 minutes, and incubated for 24 hours at 38°C with 5 to 10 units of sterile histaminase.³ The enzyme was removed by boiling and centrifugation, and the supernatant solution of SMC was evaporated to dryness, extracted with methanol and dried. In several instances, the histamine present in the original methanol solution of SMC was adsorbed by permutit (Code and Ing, 1937). This procedure proved unsatisfactory because from 25 to 50 per cent of the active constituent as well as the histamine was adsorbed. Uniform chemical and pharmacological behaviour was shown by the 11 samples of SMC which were prepared. The average yield was 30.4 mgm. per liter of blood.

One-half to 2 mgm. of histamine-free SMC contracted the n.m., the rat duodenum, the rat uterus, and the ox carotid artery strip. Five to 10 mgm. stimulated

² The term "buffy coat" is used in this paper to refer to the layer of white cells and platelets overlying the red cells in centrifuged blood which has been prevented from clotting. Although this definition of the term differs from that given in the standard dictionaries, it appears to be in accord with common usage.

³ The author is indebted to the Winthrop Chemical Co. for supplying injectable histaminase, T-360N. One unit destroyed 1 mgm. of histamine in 24 hours at 35°C.

the rabbit and guinea-pig ileum. The pupil of the cat responded to the intracarotid injection of 1 to 2 mgm. of SMC by a slight dilatation followed by a marked constriction which lasted for several minutes. The fact that the intravenous injection of atropine was without effect on the response of the n.m. to SMC demonstrates that the active agent is not a choline ester.

One milligram of SMC constricted the blood vessels of the perfused tail and ear of the cat. In contrast to this effect, as much as 30 mgm. had no action on the vessels of the rabbit ear. The injection of 9 to 70 mgm. of SMC into the arterial supply of the perfused kidney of the cat caused a decrease of from 9 to 58 per cent in the venous outflow. The blood flow through the capillaries and venules of the skin of the cat's ear, observed microscopically by the method of Hooker (1920), was decreased by the intracarotid injection of 5 to 16 mgm. of SMC.

The intravenous injection of 4 to 100 mgm./kgm. of SMC both before and after the injection of atropine usually caused a fall of 5 to 20 mm. Hg in the blood pressure of cats anesthetized with ether or nembutal. It also lowered the blood pressure of unanesthetized decapitate, spinal and decerebrate cats. An identical response was observed when SMC was injected centrally through a catheter inserted into the carotid artery, thus eliminating the possibility that the vasoconstrictor substance was completely destroyed by the lungs. That SMC did not contain histamine was shown by its failure to constrict the vessels of the rabbit ear, an indicator particularly sensitive to histamine. Moreover, the injection of SMC lowered the blood pressure of the rat, which, as is well known, is raised by histamine.

Some chemical properties of SMC were studied, using the n.m. as an indicator. SMC is soluble in 80 per cent ethanol or acetone, but is insoluble in ether, and in absolute ethanol or acetone. Two hours at room temperature in 0.1 N NaOH or HCl, one hour of boiling in 1 N NaOH or 1 N NaCl, or 10 minutes of boiling in 1 N HCl had no effect on its activity. However, the active agent was entirely destroyed by HNO_2 at room temperature and by 1 hour of boiling in 1 N HCl. It was not precipitated by saturated aqueous or alcoholic solutions of picric acid nor by phosphotungstic acid. The biuret test was positive. The activity of SMC was destroyed by incubation with crude potato extract containing tyrosinase (Beyer, 1941), and its destruction was inhibited by cyanide. Trypsin (Fischer Scientific "Pure") had no effect on its activity.

Six experiments were performed in which the action of SMC was compared quantitatively with that of a dialysate of cat serum on three indicators: the n.m., rat intestine, and perfused cat tail. The dialysate was employed rather than whole serum in order to avoid effects attributable to viscosity or to foreign proteins; its action was similar to that of the original serum. It was prepared by dialysing serum against an equal volume of Ringer solution overnight in the cold and neutralizing it with HCl. When doses of serum dialysate and of SMC dissolved in Ringer solution which gave responses of equal magnitude on one indicator were used, the responses of the other two indicators to the two substances given in the same ratio were also equal (table 4). These results suggest that a

single substance is responsible for the activity of SMC on the three types of smooth muscle, and that this substance is present both in SMC and in a dialysate of serum. Further evidence that the effect of SMC on the n.m., rat intestine and the tail vessels of the cat is produced by a single substance is the finding that

TABLE 4

A comparison of the action of cat serum dialysate and of SMC on the rat intestine, nictitating membrane, and the blood vessels of the cat tail and ear

EXPT.	INDICATOR	DIALYSATE		SMC	
		cc.	response*	mgm.	response*
1	N.M.	0.25	18	1.0	17
		0.125	13	0.5	12
	Intestine	0.5	7	2.0	11
2	N.M.	0.25	8	2.0	9
	Intestine	0.25	5	2.0	4
		0.125	0	1.0	0
	Cat ear	0.25	76	2.0	54
		0.125	64	1.0	54
3	N.M.	0.25	22	1.0	35
		0.125	14	0.5	12
	Intestine	0.9	29	3.6	16
		0.5	8	2.0	8
	Cat tail	0.125	62	0.5	65
4	N.M.	0.5	12	1.0	12
		0.25	6		
	Intestine	0.5	12	1.0	10
				2.0	25
	Cat tail	0.5	77	1.0	67
5	N.M.	0.5	10	2.0	17
	Intestine	0.25	8	1.0	18
		0.25	24	1.0	35
	Cat tail	0.5	0†	2.0	0†
6	N.M.	0.125	18	0.5	18
	Intestine	0.5	8	2.0	7
	Cat tail	0.125	59	0.5	69

* Response of n.m. and intestine is measured in mm. Response of perfused tail and ear is measured in per cent reduction of outflow, so that 0 represents the absence of vasoconstriction.

† One-half cubic centimeter of 1:1,000,000 adrenalin caused complete cessation of flow.

1 hour of boiling in 1 N HCl completely abolished the action of SMC on all three indicators, whereas boiling in 1 N NaCl or in 1 N NaOH did not impair its action on any of them.

The destruction by tissues of the active material in serum and SMC. The destruc-

tion by the perfused lungs of the substance active on the n.m. was studied in two experiments. Two cubic centimeters of defibrinated cat blood which had been perfused through the lungs of a cat for two hours produced no contraction of the n.m., whereas the injection of 1 cc. of unperfused defibrinated blood caused a marked response. The perfused blood gradually regained its activity so that its effect 2½ hours after the end of perfusion was about one-half that of the control sample.

Further experiments on the destruction of the active material were carried out by incubating 10 cc. of cat serum or 5 to 15 cc. of Ringer solution containing SMC with 1 to 5 grams of finely divided cat or rat tissue. After the



Fig. 1

Fig. 2

Fig. 1. Comparison between the activity of a dialysate of cat serum (C.S.D.) and SMC on three indicators. A. Outflow of perfused cat tail. 1, 0.5 mgm. SMC. 2, 0.125 cc. C.S.D. B. Cat nictitating membrane. 1, 0.25 cc. C.S.D. 2, 1 mgm. SMC. C. Rat intestine. 1, 0.5 cc. C.S.D. 2, 2 mgm. SMC. Time interval is 5 seconds.

Fig. 2. Response of the cat nictitating membrane. 1, 0.5 cc. extract of Ringer solution incubated with cat skeletal muscle (10 cc.: 1 gram). 2, Ringer solution. 3, 0.5 cc. extract of serum incubated with cat skeletal muscle (10 cc.: 1 gram). 4, 2 cc. extract of serum incubated with cat lung (10 cc.: 1 gram). 5, 0.5 cc. extract of serum mixed with lung tissue just before extraction. Time interval is 5 seconds.

mixtures had been shaken at 38°C for 3 hours, the toxic tissue proteins were precipitated by decanting the solutions either into 2 volumes of acetone or into 10 volumes of 95 per cent ethanol. The acetone filtrates were evaporated on a steam bath, the ethanol filtrates evaporated in vacuo, and both were made to the original volume with distilled water. In some experiments, the active agent was incubated with tissue extracts. To obtain the extracts, 1 to 5 grams of tissue were ground with sand, and mixed thoroughly with 5 to 10 cc. of Ringer solution or phosphate buffer (M/15, pH 7.4). The solid material was removed by centrifugation and the extract was incubated with 10 cc. of serum or SMC solution.

The activity of the extracts of the incubated material was tested on smooth muscle. Extracts of control samples in which Ringer solution was incubated with the tissues failed to stimulate smooth muscle, except in one experiment on

rat spleen which is omitted from table 6. The results of the incubation experiments are presented in tables 5 and 6. Four plus represents the response of the indicator to the injection of 0.5 to 2 cc. of an extract of serum or SMC incubated without the addition of tissue. The results given in the tables indicate the response to an equal volume of an extract of serum or SMC which had been incubated with various tissues.

The tissues tested, in the approximate order of their ability to destroy the activity of serum on the n.m. were: (1) cat lung and kidney, and rat lung and skele-

TABLE 5

The action on the nictitating membrane of extracts of 10 cc. of cat serum after incubation with various tissues

NO. OF EXPTS.	GRAMS OF TISSUE	TISSUE	RESPONSE*
8	1	Cat lung	0
3	1	Cat lung	++
5	1-1.5	Extract of cat lung	++
2	3-5	Extract of cat lung	++
2	4-5	Extract of cat lung	0
2	1	Rat lung	0†
5	1	Cat kidney	0
1	1	Cat kidney	++++
2	1.3-2	Cat kidney	0
1	1	Extract of cat kidney	++
1	2	Extract of cat kidney	0
1	5	Extract of cat kidney	0
4	1	Cat skeletal muscle	++++
1	5	Cat skeletal muscle	++++
2	1	Rat skeletal muscle	0†
3	1	Cat spleen	++++
1	1	Cat spleen	++
1	1.3	Cat spleen	++

* The action of an extract of 10 cc. of serum incubated without the addition of tissue is taken as ++++.

† In both experiments, the extract also failed to act on rat intestine. In 1 experiment, it caused a very slight constriction of the blood vessels of the perfused cat tail, and in the second experiment had no effect on this indicator.

tal muscle, (2) extract of cat kidney, (3) cat spleen and extract of cat lung, (4) cat skeletal muscle. A similar list compiled from the experiments on the destruction of the material in SMC which stimulates the n.m. is (1) cat lung and rat lung, kidney, liver and intestine, (2) rat skeletal muscle, cat kidney and extract of cat kidney, (3) rat spleen and extract of cat lung. The destruction of SMC by rat lung and skeletal muscle depends at least indirectly on an oxidative process, since no loss of activity occurred when the incubation was carried out in an atmosphere of nitrogen.

The results of experiments on the destruction by tissues of the activity of serum and SMC on the rat intestine and vessels of the perfused cat tail are given in the footnotes to tables 5 and 6. It was found that the activity of serum on these

TABLE 6

The action on the nictitating membrane of extracts of SMC after incubation with various tissues

NO. OF EXPTS.	MGM. OF SMC	GRAMS OF TISSUE	TISSUE	RESPONSE
3	10-30*	2	Cat lung	0
2	10-30*	2	Extract of cat lung	++++
4	12	0.5	Rat lung	0†
2	12	0.5	Rat lung	++++‡
1	27	0.43	Rat lung	±§
1	6*	1	Cat kidney	0
1	30*	7	Cat kidney	+++
1	6*	1	Extract of cat kidney	0
1	30*	7	Extract of cat kidney	++
1	12	0.5	Rat kidney	0
1	10	0.6	Rat skeletal muscle	++†
2	12	0.5	Rat skeletal muscle	++
1	12	0.5	Rat skeletal muscle	++++‡
1	27	0.43	Rat skeletal muscle	++++§
2	12	0.5	Rat spleen	++++†
1	12	0.5	Rat spleen	++
3	12	0.5	Rat liver	0†
2	12	0.5	Rat intestine	+

* Approximate amount.

† In one experiment, a similar response was also observed on the rat intestine.

‡ Incubated under nitrogen.

§ Not tested on the n.m. but on the rat intestine.

TABLE 7

The action of cat serum and SMC on smooth muscle and the effect of incubation with tissues or of treatment with acid and alkali on their activity

TEST SUBSTANCE	TREATMENT	RESPONSE OF INDICATORS			
		N.M.	Rat intestine	Cat tail vessels	Rabbit ear vessels
Serum.....		++++	++++	++++	++++
SMC.....		++++	++++	++++	0
Serum.....	Cat lung	0			
Serum.....	Rat lung	0	0	0	
SMC.....	Cat lung	0			
SMC.....	Rat lung	0	0		
Serum.....	Cat kidney	0			
SMC.....	Cat kidney	0			
SMC.....	Rat kidney	0			
Serum.....	Cat skeletal muscle*	++++			
Serum.....	Rat skeletal muscle*	0	0	0	
SMC.....	Rat skeletal muscle	++	++		
Serum.....	Cat spleen	+++			
SMC.....	Rat spleen	++++	++++		
SMC.....	Rat liver	0	0		
SMC.....	Rat intestine	0			
SMC.....	NaOH	++++	++++	++++	
SMC.....	HCl	0	0	0	

* Rat and cat skeletal muscle differ in their effect upon the activity of serum.

indicators as well as on the n.m. was abolished by incubation with rat lung and skeletal muscle. The action of SMC on the rat intestine was not diminished after incubation with rat spleen or with small quantities of skeletal muscle, but was abolished by incubation with rat liver, lung and a large amount of skeletal muscle.

A comparison of the activity of serum and SMC on smooth muscle, and experiments concerned with the destruction of the active material in serum and SMC by tissues or chemical agents are summarized in table 7.

DISCUSSION. The results of the present study demonstrate the presence in rabbit platelets of substances which act upon the smooth muscle of the rat intestine and rabbit ear vessels, and the existence in cat platelets of material which contracts the n.m. This confirms the work of earlier investigators who have shown that the platelets contain substances which stimulate the rabbit ear vessels (Reid and Bick, 1942), and the isolated intestine (T. F. Zucker and Stewart, 1913; Freund, 1920) as well as arterial strips (T. F. Zucker and Stewart, 1913; LeSourd and Pagniez, 1914; Janeway, Richardson and Park, 1918; Reid and Bick, 1942), the isolated uterus (Freund, 1920) and the vessels of the perfused frog (O'Connor, 1912; Freund, 1920). Since blood which has clotted has no greater activity on the n.m., intestine or rabbit ear vessels than do the lysed cells from an equal volume of blood, coagulation must release the active constituents from the platelets without increasing their amount. Coagulation plays a similar rôle in the release of the material which stimulates the isolated carotid artery (Reid and Bick, 1942). There is no evidence that the white cells contribute active substances to blood, since disintegrated neutrophils fail to stimulate the smooth muscle of the arterial strip (Janeway et al., 1918), and since lysed lymphocytes do not contract the n.m. or rat intestine.

The common origin from the blood platelets of the active substances, and the similarity of some of the chemical properties of these substances have led several investigators (O'Connor, 1912; Greeley, 1929) to assume that a single substance is responsible for the activity of serum on all smooth muscle indicators. It will subsequently be shown that this assumption is erroneous. However, two lines of evidence indicate that a single substance accounts for the effect of serum on the cat n.m., rat intestine and on the vessels of the perfused cat tail. First, the ratio of the activity of an acetone extract of serum to that of whole serum is the same on each of the three indicators, which might not be the case if more than one substance active on these indicators were present in the serum. Secondly, the incubation of serum with rat lung or with skeletal muscle destroys its activity as tested on any one of the three indicators. Similar experiments indicate that a single substance is responsible for the activity of a fraction prepared from the buffy coat of human blood (SMC) on the n.m., rat intestine and cat tail vessels. First, quantities of SMC and of a dialysate of cat serum which produce contractions of equal magnitude on the n.m. also produce equal responses of the smooth muscle of the rat intestine and cat tail vessels. Secondly, incubation of SMC with rat lung, liver or large amounts of skeletal muscle, but not incubation with spleen or small amounts of skeletal muscle, destroys its activity as tested on

either the n.m. or rat intestine. SMC which has been treated with acid fails to stimulate the n.m., intestine or cat tail vessels, whereas alkali-treated SMC retains all of its activity on the three effectors.

The substance in SMC which stimulates the n.m., intestine and cat tail vessels is identical with the material in serum which affects these indicators. This is demonstrated by the fact that the activity of a dialysate of cat serum bears the same ratio to the activity of SMC when it is tested on any one of the three indicators, and by the finding that the tissues which destroy the effect of SMC also abolish the activity of serum, whereas those tissues which fail to impair the activity of SMC do not affect that of serum. Although the above evidence is circumstantial in nature, it will be assumed in the remainder of this paper that a single substance, present in both serum and in SMC, is responsible for their activity on the n.m., rat duodenum and on the vessels of the cat tail.

The substance which stimulates the n.m., intestine and cat tail vessels is a dialysable, heat stable compound, possibly containing one or more amino and phenolic or catechol groups. Its failure to raise the blood pressure of the cat, which confirms the results of Zipf and Hülsmeier (1933), and its stimulant action on the intestine demonstrate that it is not a sympathomimetic amine. According to Freund (1936), tyramine is responsible for the activity of serum on smooth muscle. A consideration of the pharmacological properties of this drug indicates that it is not identical with the substance which stimulates the n.m., rat duodenum and cat tail vessels. In agreement with the conclusions of Freund (1920), Guttentag (1931), Zipf and Hülsmeier (1933), and Wu et al. (1942), it was found that the gut-stimulating substance is neither a choline ester nor histamine.

Although the SMC fraction contracts strips of carotid artery, the constituent in SMC which acts upon this indicator may prove to be separate from the factor which stimulates the intestine, n.m. and cat tail vessels. This is suggested by the observation that certain serum extracts stimulated arterial strips but not the intestine (Stewart and T. F. Zucker, 1913), and by the finding that the incubation of serum with arterial tissue abolished its effect on the former but not on the latter indicator (T. F. Zucker and Stewart, 1913).

Perfusion of defibrinated blood through the lungs abolishes its activity as tested on the uterus (Sibul, 1935), intestine (Greeley, 1929), renal vessels (Bing, 1941), and n.m. To what extent this indicates the identity of the agents which stimulate these indicators is not known. Perfusion through the spleen abolished the vasoconstrictor action of defibrinated blood on the renal vessels more effectively than did perfusion through the kidney (Bing, 1941), whereas incubation with renal tissue abolishes the n.m. stimulating activity of serum more readily than does incubation with splenic tissue. This suggests either that destruction *in vitro* is effected by a mechanism different from that which occurs in perfused organs, or that the substance producing renal vasoconstriction is distinct from that which contracts the n.m.

SMC fails to affect the rabbit ear vessels, although it contains the substance which stimulates the n.m., intestine and vessels of the cat tail. Since serum and platelets constrict the vessels of the rabbit ear, they must contain a vasocon-

strictor factor which is not present in SMC. Little is known about this factor except that it is not histamine (Wu et al., 1942; Tsai et al., 1943; Tsai et al., 1944).

A fraction which, like SMC, contracts the intestine but is devoid of activity as tested on the rabbit ear vessels was prepared by Wu, Fang and Tsai (1942) from rabbit serum, either by dialysis or by ether extraction. Tsai, McBride and M. B. Zucker (1944) made a more extensive study of the properties of a fraction which was prepared from human buffy coat or from rabbit serum by extraction with ether, and which was probably identical with the fraction described by Wu et al. (1942). This fraction stimulated the n.m., rat and rabbit intestine, and cat tail vessels but had no effect on the vessels of the rabbit ear. When freed of lipids, the active constituent of the ether extractable fraction is, like SMC, insoluble in ether. Insufficient evidence is available to determine whether or not the active substance in the ether extractable fraction is identical with that in SMC. The active agents in the two fractions differ in their response to alkali, the activity of the ether extractable fraction being readily destroyed by treatment with 0.1 N NaOH, whereas the activity of SMC is unaffected by one hour of boiling in 1 N NaOH. It is of interest to note that the ether extract of rabbit serum contains only about one-tenth of the activity of the serum from which it was prepared, and that the ether extract of the buffy coat contains less than one-tenth of the activity of SMC prepared from an equal amount of buffy coat. On the other hand, the extract of rabbit serum is about ten times more active by weight than is SMC.

SUMMARY

Rabbit serum and lysed platelets stimulate the rat intestine and the vessels of the rabbit ear, and similar fractions of cat blood contract the cat n.m., whereas plasma and hemolysed red cells have at most a slight effect on these indicators. Blood which has clotted has no greater activity on the three types of smooth muscle than do the lysed cells from an equal volume of blood. Disintegrated lymphocytes are devoid of activity on the n.m. or rat intestine. These observations confirm the conclusions of earlier workers that the breakdown of the platelets in shed blood releases the substances which stimulate smooth muscle.

Lymph contracts the n.m., produces constriction of the rabbit ear vessels and inhibits the tonus and contractions of the rat duodenum.

The preparation of a pharmacologically active fraction from the buffy coat of human blood (SMC) is described. This fraction stimulates the smooth muscle of the intestine, uterus, carotid artery, n.m., pupillary sphincter, and vessels of the cat tail, but fails to affect the blood vessels of the perfused rabbit ear. A single substance appears to be responsible for the activity of SMC on the rat intestine, n.m. and cat tail vessels. The identity of this heat stable, dialysable substance is unknown; it is not histamine, tyramine, a choline ester nor a sympathomimetic substance.

Incubation with cat lung or kidney, or with rat lung or skeletal muscle destroys the effect of serum on the n.m., whereas incubation of serum with cat spleen or skeletal muscle fails to diminish its action on this indicator. The substance in SMC which stimulates the n.m. is destroyed by incubation with cat lung or rat

lung, kidney, liver, intestine or skeletal muscle and by prolonged boiling in 1 N HCl, but not by incubation with rat spleen nor by boiling in 1 N NaOH. The activity of SMC or serum on the rat intestine and cat tail vessels is affected by incubation with tissues or by treatment with acid and alkali in the same manner as is its activity on the n.m.

The ratio of the activity of a dialysate of cat serum to that of SMC on the n.m. is identical with the ratio of their activities as tested on the rat duodenum and cat tail vessels. From this and from the similar effects of incubation with various tissues on the activity of serum and SMC, it is concluded that the substance in SMC which stimulates the n.m., intestine and vessels of the cat tail is responsible for the activity of serum on these indicators. The evidence for the existence of other smooth muscle stimulating substances in serum is discussed.

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REFERENCES

- AMBERSON, W. R. *Biol. Rev.* **12**: 48, 1937.
 BEYER, K. H. *J. Pharmacol. and Exper. Therap.* **71**: 151, 1941.
 BING, R. J. *This Journal* **133**: 21, 1941.
 CHARGAFF, E., F. W. BANCROFT AND M. STANLEY-BROWN. *J. Biol. Chem.* **116**: 237, 1936.
 CODE, C. F. AND H. R. ING. *J. Physiol.* **90**: 507, 1937.
 DALY, I. DE B., P. FOGGIE AND G. VON LUDÁNY. *Quart. J. Exper. Physiol.* **26**: 235, 1937.
 EICHHOLTZ, F. AND E. B. VERNEY. *J. Physiol.* **59**: 340, 1924.
 FREUND, H. *Arch. f. exper. Path. u. Pharmakol.* **86**: 266, 1920.
 FREUND, H. *Arch. f. exper. Path. u. Pharmakol.* **180**: 189, 1936.
 GADDUM, J. H. *Gefässerweiternde Stoffe der Gewebe*. Georg Thieme Verlag, Leipzig, 1936.
 GREELEY, H., JR. *This Journal* **90**: 705, 1929.
 GUTTENTAG, O. E. *Arch. f. exper. Path. u. Pharmakol.* **162**: 727, 1931.
 HOOKER, D. R. *This Journal* **54**: 30, 1920.
 JANEWAY, T. C., H. B. RICHARDSON AND E. A. PARK. *Arch. Int. Med.* **21**: 565, 1918.
 LESOURD, L. AND P. PAGNIEZ. *Compt. rend. Soc. de biol.* **76**: 587, 1914.
 LANDIS, E. M., J. E. WOOD AND J. L. GUERRANT. *This Journal* **139**: 26, 1943.
 O'CONNOR, J. M. *Arch. f. exper. Path. u. Pharmakol.* **67**: 195, 1912.
 REID, G. AND M. BICK. *Australian J. Exper. Biol. and M. Sc.* **20**: 33, 1942.
 RIGLER, R. *Hefters Handbuch der exper. Pharmakol. Erg.-werk.* **7**: 63, 1938.
 SIBUL, J. *Pflüger's Arch.* **235**: 742, 1935.
 STEWART, G. N. AND T. F. ZUCKER. *J. Exper. Med.* **17**: 152, 1913.
 TRENDLENBURG, P. *Arch. f. exper. Path. u. Pharmakol.* **79**: 154, 1915-6.
 TSAI, C., J. J. MCBRIDE AND M. B. ZUCKER. *Proc. Soc. Exp. Biol. and Med.* **55**: 283, 1944.
 TSAI, C., C. H. WU, H. S. FANG AND T. I. CHEN. *Proc. Chinese Physiol. Soc., Chengtu Branch* **2**: 1, 1943.
 WERLE, E. *Oppenheimer's Handbuch der Biochemie des Menschen u. der Tiere. Erg.-werk.* **3**: 1081, 1935.
 WU, C. H., H. FANG AND C. TSAI. *Proc. Chinese Physiol. Soc., Chengtu Branch* **1**: 83, 1942.
 ZIPF, K. AND P. HÜLSMEYER. *Arch. f. exper. Path. u. Pharmakol.* **173**: 1, 1933.
 ZUCKER, T. F. AND G. N. STEWART. *Zentralbl. f. Physiol.* **27**: 85, 1913.

THE RATE OF ENTRANCE OF RADIO SODIUM INTO THE AQUEOUS HUMOR AND CEREBROSPINAL FLUID

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Knowledge of the rate of entrance of sodium isotope ions into the aqueous humor and cerebrospinal fluid is of interest in connection with the problem of the mechanism of formation and the fate of those fluids. Experiments have therefore been designed and carried out to determine the rate at which Rd Na^{24} approaches equilibrium between plasma and the fluids in question when their natural rates of production and removal are not disturbed.

EXPERIMENTAL. Rd Na^{24} was prepared by deuteron bombardment of NaOH , which was subsequently converted to the chloride. This material was used 12 to 24 hours after bombardment.

In the case of the cerebrospinal fluid and aqueous humor radioactivity was determined on dried aliquots of suitable size, employing a Geiger-Mueller counter of the β -ray type, controlled with a scale-of-two circuit. In the case of plasma the samples were first ashed in some cases. Control studies have shown that ashing is unnecessary. Background counts were made for each run. The background count for the instrument employed varied from day to day between 7.1 and 8.0 impulses per minute. Blood plasma samples were of such size and activity as to yield between ten and forty times the background count, and aqueous humor samples yielded between four and ten times. Counting was for 10 to 30 minute periods, the latter with samples of lower activity. The probable error of radioactivity measurement is calculated to be not greater than ± 4 per cent. Counts for each experiment were corrected for decay to zero counting time.¹

The movement of sodium from the blood into the aqueous humor and the cerebrospinal fluid has been studied in dogs of 10 to 20 kgm. after intravenous injection of suitable amounts of the radio isotope as sodium chloride in 25 to 50 cc. isotonic solution. After injection the blood level of Rd Na^{24} was determined on samples of arterial blood drawn at intervals by clean dry syringe from the intact femoral artery. The blood was rendered incoagulable with oxalate, and plasma was employed for analysis. At various times after injection the aqueous humor was carefully withdrawn from each eye into a clean dry tuberculin syringe through a no. 25 gauge needle inserted at the sclera-corneal border. In each case the eye was untouched before sampling and employed only once. It is well known that the composition of fluid drawn after previous draining differs markedly in composition from the normal aqueous (1). Cerebrospinal fluid was

¹ We are indebted to Dr. John H. Williams of the Department of Physics for the preparation of Rd Na^{24} , and to Dr. Wallace D. Armstrong of the Division of Physiological Chemistry for advice and assistance in the radioactivity measurements.

withdrawn from the cisterna magna; 5 cc. samples were ordinarily withdrawn. In one instance two such samples drawn consecutively showed identical radioactivity.

Observations have been made on eight dogs, five under nembutal anesthesia administered intraperitoneally, and three employing 1 per cent cocaine as a local anesthetic for paracentesis. The results of a typical experiment under nembutal are presented in table 1. It will be noted that the blood level of Rd Na²⁴ did not alter after 32 minutes, and that at 39 minutes after injection the radioactivity of the aqueous humor was 67 per cent as great as that of the plasma. A few minutes later the cerebrospinal fluid sampled from the cisterna magna showed less than half as much Rd Na²⁴. The second eye, previously untouched, was sampled at a later time and showed a closer approach to the plasma level.

The results of 16 observations on the aqueous humor and five upon the cerebrospinal fluid are summarized in figure 1. In each case the values for the two eyes

TABLE 1

Rd Na²⁴ expressed as impulses per cubic centimeter per minute corrected to zero counting time

TIME	BLOOD	AQUEOUS HUMOR	CEREBRO-SPINAL FLUID	PER CENT APPROACH TO BLOOD PLASMA LEVEL
<i>min.</i>				
0	Injection			
5	143.5			
10	101.9			
24	98.4			
32	91.7			
39		62.4 left eye		67
43			27.8	30
43.5			28.6	31
48	92			
51		74.4 right eye		81

are connected. It will be noted that in the five upper lines, which describe the results in the experiments upon nembutal anesthetized dogs, there is great uniformity, indicating an approach toward equality of Rd Na²⁴ concentrations in plasma and aqueous at something over an hour after injection on the average. These rates of approach to equilibrium are much more rapid than those observed when other methods of study are employed (2).

In the other three experiments employing local anesthesia there is decidedly less regularity and furthermore the rate of Rd Na²⁴ entrance seems to be definitely lower. Five of the six observations fall at points lower than any at similar times in the nembutalized animals, and also below any extended curve for the two eyes of any such animals. It is not impossible that autonomic nervous influences played a part in this rather consistent difference.

It may be noted that the entrance of Rd Na²⁴ into the cerebrospinal fluid was much slower than into the aqueous. The rate of entrance is from 25 to 40 per cent as great.

DISCUSSION. The observations reported above represent fundamentally measurements of the rate at which a particular cation enters two fluid compartments in the body, from the blood. If it be assumed, as seems justified, that Rd Na^{24} ions have properties essentially similar, except for radioactivity, to those of Na^{23} ions, the rate of Rd Na^{24} movement may be taken as a measure of the rate of turnover of total Na^+ .

If one assumes that there is no specific active transport of Na^+ such exchange might be of two general sorts. There is first the possibility that the movement

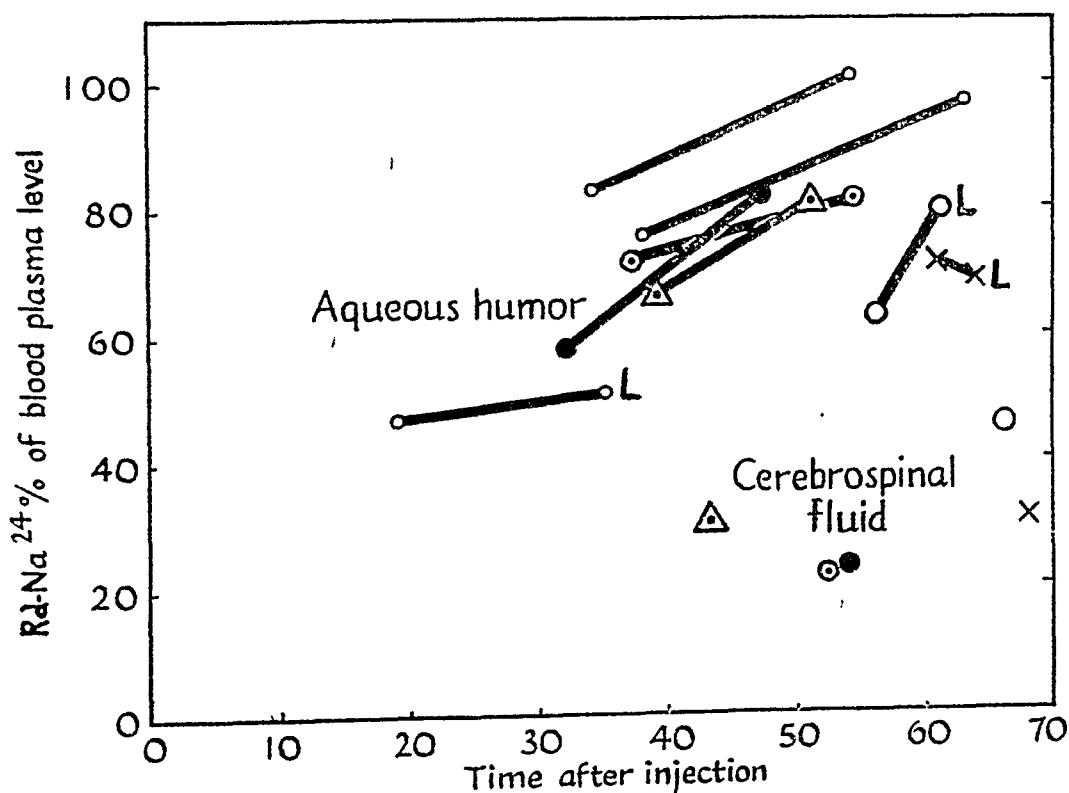


Fig. 1. Radioactive sodium concentration in the aqueous humor and cerebrospinal fluid of dogs in per cent of the simultaneous plasma level as a function of time after Rd Na^{24} injection. Points connected by lines represent aqueous humor from the two eyes of a single dog withdrawn without previous disturbance of the eye. Separate points indicate cerebrospinal fluid values. The point symbols are the same for the two fluids from each animal. The experiments marked *L* indicate local anesthesia for paracentesis. All other animals were under nembutal.

represents simple diffusion exchange. A second alternative is that the fluids in question are formed anew by ultrafiltration from the plasma, and reabsorbed, at such rates as to lead to the results observed. There is, moreover, no obvious reason for excluding the occurrence of both processes simultaneously.

If the rate of formation of the fluids in the aqueous humor and cerebrospinal fluid compartments were very slow, as compared with the times here considered, the rate of entrance of Rd Na^{24} into the compartments would constitute a measure of the permeability of the structures lining them to Na^+ . On the other hand, if the walls of the compartments, aside from the special structures for their forma-

tion and absorption, were impermeable, or nearly so, to the ion in question the rate of entrance of that ion would constitute a measure of the rate of replacement of the fluid in the compartment; in other words, a measure of the rate of formation and reabsorption of that fluid at the special sites for those processes.

The further possibility exists that there is an active and specific transport mechanism for certain constituents of these fluids. For the case in point one could conceive of a transport of a sodium salt with greater or less than isotonic equivalents of water, and with various proportions of other plasma crystalloid constituents.

The rapidity of entrance of $Rd\ Na^{24}$ into the aqueous humor could be accounted for by any one or a combination of the following assumptions: 1, that the walls of the chambers containing it are freely permeable to Na^+ ; 2, that there is a very rapid turnover of the total fluid, assuming the latter to be virtually an ultrafiltrate; 3, or that a specific mechanism exists for moving sodium salts rapidly. Without other information it is impossible to state which one, or which combination, of factors is responsible for the results. Similar considerations apply in general to the case of the cerebrospinal fluid, except for the differences in the geometry of the chambers containing it. Our observations in this connection are too scanty to warrant more than the recording of the results obtained.

The evidence at present available concerning the mechanism of aqueous humor formation is contradictory. It is generally agreed that several non-electrolyte crystalloids are present in significantly lower concentration in the aqueous than in the plasma (3). Such evidence is, however, inadmissible as proof of the occurrence of active processes in the formation of the aqueous. Obviously differences in the passive permeability of the membranes involved, with respect to such crystalloids, could account for the findings observed. The concentrations of the diffusible ions in plasma and aqueous humor are not in perfect agreement with prediction for thermodynamic equilibrium, but the differences are not great (4). However, a consistent difference in total osmotic pressure as measured by vapor tension methods has been observed by Roepke and Hetherington (5), as between blood plasma and aqueous humor, simultaneously drawn. The hyperosmotic state of the aqueous is reduced or abolished by intraocular injection of small quantities of $HgCl_2$. These observations are not in harmony with the more simple theories of the mechanisms of formation and absorption of the aqueous. Moreover, aqueous humor is absorbed into the blood against venous pressures higher than the intra-ocular pressure, according to Friedenwald and Pierce (6). Thus, there are at least two lines of evidence indicating that passive processes are inadequate to account for the character and the fate of the aqueous humor. The experiments reported in this paper add new information concerning these problems but do not permit one to choose between the three general alternatives listed above. Since this paper was prepared a report (7) has appeared in which similar observations are described in another animal species.

SUMMARY

The rate of movement of $Rd\ Na^{24}$ into the aqueous humor and cerebrospinal fluid has been measured. The use of radioactive isotopes of important naturally

occurring constituents provides a measure of rates of movement without significant alterations in total ionic concentrations, osmotic pressure or hydrostatic pressure. The rate of entrance observed is therefore a measure of normal rates of exchange or turnover. In the anesthetized dog the Rd Na^{24} concentration in the aqueous reaches about 75 per cent of the plasma level 45 minutes after its intravenous injection; this represents a much more rapid rate of turnover than has been indicated previously by transport studies involving concentration differences between plasma and aqueous. The rate of entrance was found to be somewhat slower in unanesthetized animals. In anesthetized animals the rate of approach to equilibrium between plasma and the cerebrospinal fluid sampled from the cisterna magna is 25 to 40 per cent as great as in the case of the aqueous humor.

REFERENCES

- (1) DUKE-ELDER, S. Nature of the intra-ocular fluids, 1927.
- (2) DAVSON, H. AND J. P. QUILLIAM. J. Physiol. 98: 141, 1940.
- (3) ROBERTSON, J. D. AND P. C. WILLIAMS. J. Physiol. 95: 139, 1939.
- (4) DUKE-ELDER, S., J. C. QUILLIAM AND H. DAVSON. Brit. J. Ophthalmol. 24: 421, 1940.
- (5) ROEPKE, R. R. AND W. A. HETHERINGTON. This Journal 130: 340, 1940.
- (6) FRIEDENWALD, J. S. AND H. F. PIERCE. Arch. Ophthalmol. 7: 538, 1932; 8: 9, 1932.
- (7) KINSEY, V. E., W. M. GRANT, D. G. COGAN, J. J. LIVINGOOD AND B. R. CURTIS. Arch. Ophthalmol. 27: 1126, 1942.

THE INFLUENCE OF TEMPERATURE ON SPINAL CORD DAMAGE CAUSED BY ASPHYXIATION

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The effects of asphyxiation of the caudal part of the spinal cord on the reflex activity in hind legs and tail, on the metabolism of the cord and on its histology have been described in previous papers (1, 2, 3). In these experiments the cord was asphyxiated at the normal body temperature. In order to examine the temperature influence on the damage caused by cord asphyxiation, the results of asphyxiation at normal body temperature were compared in the present investigation with those at and about 10° lower temperature.

METHOD. The spinal cord was asphyxiated by forcing Ringer solution into the isolated caudal part of the dural cavity under a pressure higher than the blood pressure (26–28 cm.), thus interrupting the blood circulation in the cord (1). The caudal part of the cord and dural cavity were isolated the day preceding the asphyxiation by ligating the dura at Th 10–12 under aseptic precautions. As in previous experiments (1, 2, 3) cats were used.

In the "cold" experiments the lightly anesthetized (nembutal) animal was cooled for 20 to 30 minutes in ice water until its temperature had dropped to 27–28° (rectal). During the period of asphyxiation the cat's temperature was kept as constant as possible by evaporating alcohol on the skin or by applying hot water bottles as needed. It was possible in this way to keep the variations of the body temperature within 1° even during an asphyxiation of two hours' duration. The room in which the experiments were performed and the sterile Ringer solution used for the asphyxiation were heated to the expected body temperature (27–28°). The Ringer solution thus entered the dural cavity at the lowered body temperature. After the cord asphyxiation the animal was heated to 37–38° in 30 to 45 minutes. The cat's temperature was then watched for the ensuing two to three hours until the temperature regulation had recovered sufficiently to maintain a normal body temperature.

In the "warm" experiments the body temperature was maintained at about 38° by the same means as described for the "cold" asphyxiations. The room and the Ringer solution again were heated to the cat's body temperature and the Ringer solution thus entered the dural cavity at a temperature of about 38°.

The reflex activity in the hind legs and tail was noted for a period of 5 days after asphyxiation, then the animal was sacrificed for the metabolic and histologic studies of the cord. This period was selected because it was known from previous experiments that though large changes in reflex activity, metabolism and histological structure take place during the first two days, no important changes occur later than 3 to 4 days after asphyxiation.

The spinal cord caudal of L5 was removed for the metabolism determination. It was divided into four parts: the 6th and 7th lumbar segments, the first sacral segment and the segments caudal of S1. The oxygen consumption of each of these parts was determined in Warburg respirometers at 38° in the way previously described (2).

The 5th lumbar segment was fixed in 95 per cent alcohol, further dehydrated, embedded in paraffin and after cutting (15 μ), stained with toluidine blue.

TABLE 1
Thirty-five minutes' cord asphyxiation

	NUMBER ANIMAL	TEMPERATURE ANIMAL	REFLEXES 5 DAYS AFTER ASPHYXIATION				METABOLISM mm. ³ /100 mgm.	CELLS PER HORN IN L5
			Kneejerk	Extensor tone	Flexion reflex	Tail reflex		
A	1	37.9	—	(+)	—	+	37	6
	2	37.7	—	—	—	—	42	12
	3	38.0	(+)	+	—	+	42	13
	4	38.6	—	—	—	(+)	36	2
	5	38.1	—	—	—	—	46	3
	6	38.6	—	—	—	(+)	49	9
Average		38.2					42	8
B	7	28.5	+++	—	++++	+++	66	46
	8	27.0	++++	—	+++	+	63	29
	9	27.7	++++	—	+++	+	68	33
	10	27.1	+++	—	+	(+)	64	36
	11	27.8	+++	—	++++	++++	66	31
	12	26.7	+++	—	+++	+	71	38
Average		27.5					66	35

The figure given for the animal's temperature is the average of temperature readings at 5 minute intervals during the period of asphyxiation. One plus indicates a slight, two pluses a brisk and three pluses a markedly hyperactive kneejerk, flexion and tail reflex. One, two or three pluses indicate the intensity of exaggerated extensor tone. Three pluses are given when the leg can be bent only with difficulty. The average O₂ consumption of the cord caudal of L5 is expressed in mm.³/100 mgm. of wet tissue/hour. The cell number indicates the number of cells in one anterior horn in a 15 μ thick section of the 5th lumbar segment.

RESULTS. A. *Effect of 35 minutes' asphyxiation.* In table 1A the results of six 35 minute asphyxiations at about 38° are listed. The reflex activity 5 days after asphyxiation was found to be seriously impaired. In none of the animals could the flexion reflex be elicited; in only one cat was a trace of kneejerk present. In two cats some extensor tone was found. In some of the animals small movements of the tail could be elicited by pinching.

The figures for the metabolism give the average oxygen consumption of the spinal cord caudal of L5 in mm.³/100 mgm. of wet tissue/hour. This figure was computed from the O₂ consumption and the weights of the 4 parts into which the

cord was divided for the metabolism determination. The gradient of metabolism found previously (2) in this part of the spinal cord was observed again, the more caudal parts of the spinal cord having a higher oxygen consumption than the more cranial ones. The oxygen consumption of the cord caudal of L5, 3 to 14 days after ligation of the dura at Th 10-12 but not subjected to asphyxiation has been determined before (2) and was found to be about $65 \text{ mm.}^3/100 \text{ mgm. of wet tissue/hour}$. This figure is taken as the control value for all the present experiments. Five days after the 35 minutes' asphyxiation at about 38° an average O_2 consumption of 42 mm.^3 was found which is considerably below the control value and which is in agreement with previous determinations under similar circumstances (2).

The large nerve cells in the anterior horns were counted in 20 sections of the 5th lumbar segment. From this an average number of nerve cells in one 15μ thick section of one anterior horn was computed. In 14 normal cats 40 ± 1 cells were found per section of one anterior horn. After 35 minutes' asphyxiation at 38° the number of nerve cells was much smaller; an average of only 8 cells per horn was found.

In table 1B the results of six 35 minute asphyxiations at an average temperature of 27.5° are given. The effects of such an asphyxiation are quite different. In all the animals a brisk kneejerk was present. A pronounced flexion reflex, accompanied by crossed extension was found in all animals. In some cats the excitability of these reflexes was considerably higher than normally found in a spinal animal. None of these animals showed exaggerated extensor tone. In all 6 cats the tail reflex was present. The average oxygen consumption of the cord caudal of L5 was 66 mm.^3 , which is not different from the control value. The average of 35 nerve cells per anterior horn was slightly below the control value.

B. Effect of 60 minutes' asphyxiation. The effects of 6 experiments in which the spinal cord was asphyxiated for 60 minutes at about 38° are collected in table 2A. No reflex activity at all was found 5 days after asphyxiation. The cord metabolism had dropped to 32 mm.^3 and in only two animals a few nerve cells were found in the anterior horns. These results are in agreement with the effects of a 60 minute asphyxiation at normal body temperature observed before (2).

In table 2B are found the results of six 60 minute "cold" asphyxiations. Considerable reflex activity was observed 5 days after asphyxiation. The flexion reflex, which was sometimes accompanied by crossed extension, was often hyperactive, making it difficult or even impossible to examine the kneejerk since attempts to elicit this reflex resulted in contractions of the flexion muscles of the leg. In other animals the kneejerk could be elicited without difficulty. The tail reflex was present in all animals. No exaggerated extensor tone was found. The average metabolism of the cord caudal of L5 was 64 mm.^3 , the same as the control value. An average of 36 cells was found in the anterior horns which is slightly below the number found in the normal 5th lumbar segment.

C. Effect of 90 minutes' asphyxiation. Table 3A shows the results of six 90 minute asphyxiations at about 38° . As in the 60 minute experiments no reflexes

TABLE 2
Sixty minutes' cord asphyxiation

	NUMBER ANIMAL	TEMPERATURE ANIMAL	REFLEXES 5 DAYS AFTER ASPHYXIATION				METABOLISM	CELLS PER HORN IN L5
			Kneejerk	Extensor tone	Flexion reflex	Tail reflex		
A	13	38.5	—	—	—	—	30	0
	14	38.9	—	—	—	—	30	0
	15	38.0	—	—	—	—	33	2
	16	38.0	—	—	—	—	30	2
	17	37.5	—	—	—	—	34	0
	18	38.5	—	—	—	—	32	0
Average		38.2					32	
B	19	27.4	+++	—	+++	+	67	40
	20	27.8	*	—	+++	++	68	41
	21	27.1	++	—	+	(+)	59	26
	22	27.6	++	—	++	+	61	38
	23	27.1	*	—	+++	+	64	35
	24	26.3	+	—	++	++	63	37
Average		27.2					64	36

* In these cases the flexion reflex was so hyperactive that any attempt to elicit the knee-jerk resulted in flexion of the leg.

See note below table 1.

TABLE 3
Ninety minutes' cord asphyxiation

	NUMBER ANIMAL	TEMPERATURE ANIMAL	REFLEXES 5 DAYS AFTER ASPHYXIATION				METABOLISM	CELLS PER HORN IN L5
			Kneejerk	Extensor tone	Flexion reflex	Tail reflex		
A	25	37.5	—	—	—	—	39	0
	26	37.7	—	—	—	—	33	0
	27	38.9	—	—	—	—	29	0
	28	38.0	—	—	—	—	38	0
	29	37.8	—	—	—	—	40	0
	30	38.4	—	—	—	—	30	0
Average		38.1					35	
B	31	27.8	†	+++	(+)	+++	51	15
	32	27.5	++	—	++	++	58	40
	33	26.6	++	+	++	+	53	38
	34	26.8	*	+	+++	+	50	31
	35	27.1	+++	—	++	+++	61	33
	36	27.6	*	—	+++	++	55	32
Average		27.2					55	32

* In these cases the flexion reflex was so hyperactive that any attempt to elicit the knee-jerk resulted in flexion of the leg.

† The extensor tone was so high that the kneejerk could not be examined.

See note below table 1.

were present 5 days after asphyxiation. No nerve cells were found in the anterior horns. The O_2 consumption in some animals was slightly higher than in the 60 minute experiments. A marked proliferation of the interstitial cells was found in some of these cords which may account for this higher metabolism.

The results of six 90 minute asphyxiations at about 27° are given in table 3B. Considerable reflex activity was still present 5 days after asphyxiation. Though no crossed extension was observed, the flexion reflex could be elicited in all the animals. This reflex was so hyperactive in two cats that it masked the kneejerk. In three cats exaggerated extensor tone was present. In one animal this tone was so high that the kneejerk could not be examined. In three cats hyperactive kneejerks could be elicited. The tail reflex was present in all cases, in some it was hyperactive. The cord metabolism showed an average of 55 mm.³, which is definitely below the control value. The number of cells found in the anterior

TABLE 4
One hundred twenty minutes' cord asphyxiation

NUMBER ANIMAL	TEMPERATURE ANIMAL	REFLEXES 5 DAYS AFTER ASPHYXIATION				METABOLISM	CELLS PER HORN IN L5
		Kneejerk	Extensor tone	Flexion reflex	Tail reflex		
37	27.3	—	—	—	—	42	4
38	27.7	++	—	+++	+	55	34
39	27.5	+	++	+	+	57	18
40	27.4	—	—	—	—	44	4
41	26.3	—	+	+	(+)	44	28
42	27.2	+	++	++	+	49	12
Average..	27.2					49	17

See note below table 1.

horns (32 cells) was reduced as compared with the number found in the 60 minute "cold" experiments.

D. *Effect of 120 minutes' asphyxiation.* The results of six 120 minute "cold" asphyxiations are presented in table 4. In two animals no reflex activity was present 5 days after asphyxiation. The cords of these animals showed a low metabolism and cell count. In the other animals the flexion and tail reflexes were present. No crossed extension was seen. In some of the cats an exaggerated extensor tone was present and the kneejerk could be elicited. The metabolism and especially the cell number in the anterior horns were higher in the animals which showed reflex activity than in the two in which all reflex activity was absent.

E. *Cooling after asphyxiation at normal body temperature.* It has been found that the damage caused by asphyxiation of the cord is not apparent immediately after asphyxiation (2). Shortly after a 60 minute asphyxiation reflexes may return and the metabolism may become almost as high as before asphyxiation. After a number of hours, however, the effects of asphyxiation become noticeable;

the reflexes disappear, the cord metabolism decreases and histological changes develop. It seemed of interest to examine the effect of cooling the animal during the time that the changes in the cord appear, after asphyxiation at normal body temperature. A few cats were cooled in ice water immediately after a 35 minute asphyxiation at 38°. These animals then were kept for the next 24 hours in a water bath regulated at 28 to 30° which kept the body temperature at 30 to 31°. A light narcosis was maintained with nembutal during this period. Five days after the end of the cooling period the reflexes were noted and the metabolism and histology of the cord were examined. The reflexes and the values for metabolism and cell number were within the variations found for the six "warm" 35 minute asphyxiations collected in table 1A in which the asphyxiation was not followed by a period of low body temperature. It thus seems that once the cord is damaged by asphyxiation, cooling has no counteracting effect any more.

F. *The metabolism of the normal spinal cord at 28°.* In 6 normal cats the oxygen consumption of the spinal cord caudal of L5 was determined at 28°, ten degrees

TABLE 5
Cord metabolism at 28° in millimeter³ of O₂/100 mgm. tissue/hour

NUMBER	L6	L7	S1	TAIL	L6-TAIL
43	30	39	50	60	42
44	24	27	31	47	32
45	26	31	32	51	35
46	23	25	32	41	30
47	30	41	35	50	39
48	34	37	36	49	37
Average.....	28	33	36	50	36

The metabolism at 28° of the 6th and 7th lumbar, the first sacral segment and of the segments caudal of S1 (tail) are given. In the last column the average metabolism of the cord caudal of L5 are shown, computed from metabolism and weight of the parts.

below the temperature at which the control value was determined. The results are shown in table 5. The normal cord at 28° has an average oxygen consumption of 36 mm.³, which is slightly more than half the consumption of the cord at 38°. The gradient of metabolism in this part of the cord is well demonstrated in the table. The most caudal parts have the highest metabolism.

DISCUSSION. During the first two days following a severe damage to the spinal cord by asphyxiation, a temporary return of tendon reflexes and extensor tone has been observed, and also of the flexion reflex after slightly shorter asphyxiations (1). After less severe damage reflex activity, once it has recovered, remains for the rest of the animal's life. In the latter cases when the damage has been relatively severe a high extensor tone is frequently the most pronounced remaining reflex, though other reflexes usually have been present during the first two days after asphyxiation. When the damage has been lighter other reflexes also remain permanently. These reflexes are often hyperactive (1, 3). After short periods of asphyxiation reflex activity may return to normal.

In the 35 minute "warm" experiments the damage to the spinal cord has been quite severe. All the animals showed exaggerated extensor tone and brisk tendon reflexes during the first two days, and in some cases a flexion reflex. Five days after asphyxiation very little of this reflex activity remained. The metabolism and the number of cells in the anterior horns were greatly diminished.

The hyperactivity of the kneejerk, flexion reflex and tail reflex often observed in the 35 and 60 minute "cold" experiments indicates only a mild damage of the spinal cord which in none of the cases was severe enough to produce an exaggerated extensor tone in the leg. Though the number of cells in the anterior horn was slightly lower than in the control animals, the figures for the cord metabolism were about the same as the control value. Usually there is an increase in the interstitial cells 5 days after asphyxiation, which may account for the discrepancy between the slightly lower cell number and the normal metabolism.

After 90 and especially after 120 minutes of "cold" asphyxiation the damage to the cord is more severe. In the 90 minute experiments hyperactive reflexes are still found, but in some animals the damage to the cord has been sufficient to produce a permanent, exaggerated extensor tone. In some of the 120 minute experiments the same reflex activity was found, but in others no reflexes at all were present 5 days after asphyxiation. The average metabolism and cell counts of the 90 and even more of the 120 minute experiments are considerably below the control values.

Bass (4) found an average Q_{10} of the metabolism of about 1.6 for guinea pig spinal cord at temperatures ranging from 14° to 20° . Himwich, Bowman, Fazekas and Goldfarb (5) determined the oxygen consumption of minced rat brain over a temperature range of 25 to 45° , their figures indicating a Q_{10} of about 2.2. In the present experiments a metabolism of 36 mm.^3 was found for the normal spinal cord at 28° as compared to an oxygen consumption of 65 mm.^3 of the same part of the cord at 38° . The processes involved in the oxygen uptake of the cord thus increased about 1.8 times when the temperature was increased from 28 to 38° .

A comparison of tables 1A and 4 shows that even a 120 minute asphyxiation at a temperature of 27.2° does not damage the reflex activity of the cord as severely, nor does it reduce the metabolism and cell number, as drastically as a 35 minute asphyxiation at 38.2° . Thus the damage caused at 38° by a 35 minute asphyxiation is more severe than a 3.4 times longer asphyxiation at an 11° lower temperature. This shows that the temperature coefficient of the processes ultimately resulting in nerve cell destruction is high. Their Q_{10} is probably more than 3 and thus considerably higher than that of the processes involved in the oxygen uptake of the cord.

SUMMARY

The spinal cords of cats were asphyxiated for various times at normal body temperature and at an about 10° lower temperature.

The damage of the cord as indicated by the changes in its reflex activity,

metabolism and histological structure caused by a 35 minute asphyxiation at 38° is more severe than that after a 120 minute asphyxiation at 27°. It can be concluded that the processes causing the cord damage have a large temperature coefficient.

REFERENCES

- (1) HARREVELD, A. VAN AND G. MARMONT. *J. Neurophysiol.* **2**: 101, 1939.
- (2) HARREVELD, A. VAN AND D. B. TYLER. *This Journal* **138**: 140, 1942.
- (3) HARREVELD, A. VAN. *This Journal* **131**: 1, 1940.
- (4) WINTERSTEIN, H. *Handbuch der normalen und pathologischen Physiologie*. Berlin, Julius Springer, 1929.
- (5) HIMWICH, H. E., K. M. BOWMAN, J. K. FAZEKAS AND W. GOLDFARB. *Am. J. Med. Sci.* **200**: 347, 1940.

THE HEMOGLOBIN CONCENTRATION OF THE BLOOD OF INTACT AND SPLENECTOMIZED DOGS UNDER PENTOBARBITAL SODIUM ANESTHESIA WITH PARTICULAR REFERENCE TO THE EFFECT OF HEMORRHAGE

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Previous reports (1-6) have demonstrated conclusively that general anesthesia with certain of the barbiturate series of drugs, such as sodium amytal and pentobarbital sodium, causes a dilatation of the spleen with sequestration of red blood cells in that organ and consequently a marked decrease of the hemoglobin and red cell content of the circulating blood.

This report summarizes further observations of this phenomenon, including a study of the effect of bleeding intact and splenectomized dogs that were under pentobarbital sodium anesthesia.

METHODS. Six healthy dogs were anesthetized intravenously with 25 to 30 mgm. of pentobarbital sodium per kilogram of body weight. Samples of blood for hemoglobin determination were obtained by venipuncture immediately before anesthesia and at twenty or thirty minute intervals thereafter for three hours. The hemoglobin content was determined by the technic of Sanford and Sheard (7) using the Cenco-Sheard-Sanford photometer, all determinations being done in duplicate. All the venipunctures and determinations of hemoglobin were done by one person to minimize and standardize the personal error.

Several days after the original studies the same dogs were restudied in a similar manner except that they were bled 30 cc. per kilogram of body weight in five minutes from a cannula inserted into a brachial artery, the bleeding being done immediately after the first postanesthetic blood sample had been taken. In addition the concentration of hemoglobin was determined twenty-four hours later and the blood, which had been defibrinated, was returned to the dog. One week later the dogs were splenectomized while they were under ether anesthesia. Two to three weeks after splenectomy the foregoing studies of the blood were repeated.

RESULTS. 1. *Pentobarbital sodium anesthesia of intact dogs.* Twenty minutes after anesthesia the hemoglobin concentration averaged 79 per cent of the pre-anesthetic level (range 67 to 90). During the next hour there was a further decrease to 75 per cent (range 64 to 89). Three hours after anesthesia the concentration had risen to 83 per cent (range 74 to 106).

2. *Pentobarbital sodium anesthesia of intact dogs followed by a hemorrhage of 30 cc. per kilogram of body weight in five minutes.* Twenty minutes after anesthesia the hemoglobin concentration averaged 84 per cent of the pre-anesthetic level (range 73 to 97). Twenty-five minutes after the hemorrhage the concentration

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had risen to 90 per cent (range 83 to 105). The concentration remained approximately the same during the next three hours but after twenty-four hours it had fallen to 82 per cent (range 76 to 99).

3. *Pentobarbital sodium anesthesia of splenectomized dogs.* Twenty minutes after anesthesia the concentration of hemoglobin averaged 93 per cent of the pre-anesthetic level (range 89 to 95). There was a steady rise during the next two and a half hours and at the end of the experiment the concentration averaged 100 per cent (range 94 to 108).

4. *Pentobarbital sodium anesthesia of splenectomized dogs followed by a hemorrhage of 30 cc. per kilogram of body weight in five minutes.* Twenty minutes after anesthesia the hemoglobin concentration averaged 100 per cent of the pre-anes-

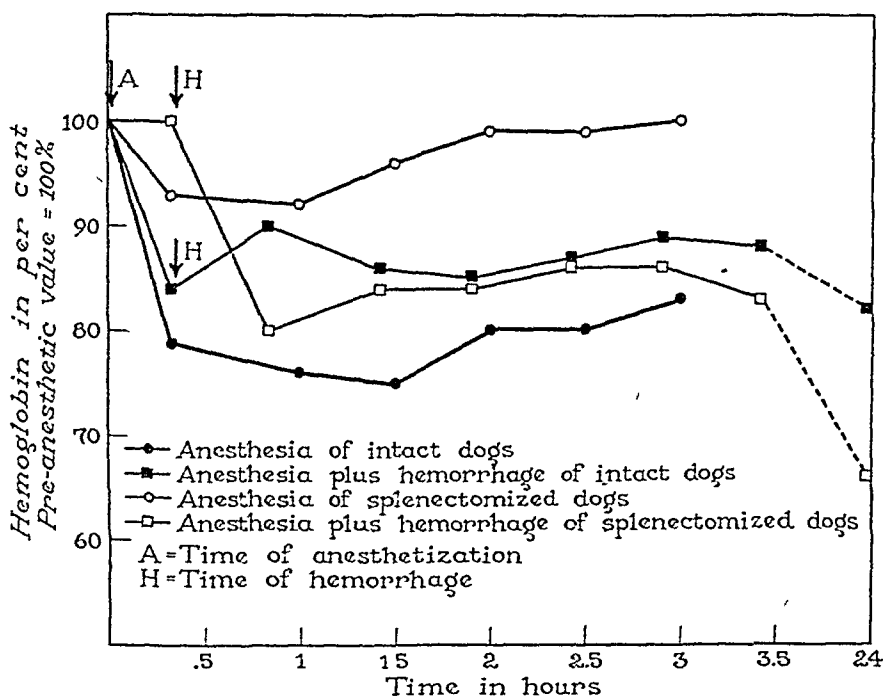


Fig. 1. Changes of the concentration of hemoglobin of the blood of intact and splenectomized dogs under pentobarbital sodium anesthesia with and without hemorrhage (30 cc. per kgm. of body weight in 5 min.).

thetic level (range 92 to 106). Twenty-five minutes after the hemorrhage the average concentration had fallen to 80 per cent (range 72 to 87). There was a slight rise during the following two and a half hours. Twenty-four hours later there had been a marked drop of the concentration, the average being 66 per cent (range 55 to 74) (fig. 1).

COMMENT. These experiments confirm the previous work showing that pentobarbital sodium anesthesia of intact animals causes a reduction of the concentration of hemoglobin and that this change is almost completely absent after splenectomy.

That dilatation of the spleen is the major cause of the decrease of concentration of hemoglobin is now an established fact. The mechanism by which barbiturate

anesthesia produces dilatation of the spleen is not known. Searles and one of us (Essex, 4) showed that denervation of the spleen did not have any effect on the dilatation that follows sodium amytal anesthesia. It has been suggested that the complete relaxation of the animal is the cause of the dilatation and Price, Hanlon, Longmire and Metcalf (8) showed that rest alone would reduce the hematocrit value 6 to 8 points. We have also observed this phenomenon. With this in mind a series of four dogs was anesthetized with pentobarbital sodium and then given strenuous passive exercise (flexing and extending the extremities and turning the dog from side to side) for twenty minutes. At this time the hemoglobin concentration had dropped to 82 per cent (range 81 to 84) of the pre-anesthetic level in spite of the exercise. After twenty additional minutes during which the dogs were at complete rest the hemoglobin concentration still averaged 82 per cent of the pre-anesthetic level (range 76 to 86). It should be noted that Cook and Rose (9) showed for cats anesthetized with sodium amytal that exercise induced by stimulating nerves with an electric current would produce a contraction of the dilated spleen.

Adolph and Gerbasi (3) pointed out that sodium amytal anesthesia caused a drop of the blood pressure and therefore would allow some migration of fluid from the tissues to the blood stream. This would account for a small part of the decrease of hemoglobin concentration. Barbour (10) demonstrated that amytal anesthesia of the rabbit caused a shift of some cellular water into the blood stream. This shift of body fluid may account at least in part for the small decrease of concentration of hemoglobin in the splenectomized dog when anesthetized with one of the drugs of the barbiturate series.

The reaction of the dogs to acute hemorrhage was quite different in the two series of experiments. The intact dogs showed a marked increase of the concentration of hemoglobin after the hemorrhage and at the end of twenty-four hours still had 82 per cent of their pre-anesthetic concentration of hemoglobin. After splenectomy the dogs showed a marked fall of concentration of hemoglobin after the hemorrhage and after twenty-four hours had only 66 per cent of their pre-anesthetic concentration of hemoglobin.

The difference of reaction to hemorrhage appears to be due to the dilated spleen acting as a reservoir of blood, high in erythrocyte concentration, upon which the body can call whenever the need arises. That this difference is of great value in protecting the intact animal against hemorrhage has been shown by Lehman and Amole (11), who compared the effects of bleeding a large series of normal and splenectomized dogs while the animals were under sodium amytal anesthesia. The dogs were bled 5 cc. per kilogram of body weight every five minutes until profound shock or death occurred. There was a more precipitous fall of the blood pressure of the splenectomized dogs and the death rate was much higher in this group at the end of thirty minutes than for the normal animals. In our series all dogs survived the loss of 30 cc. of blood per kilogram of body weight in five minutes before splenectomy. Only one of them died from such a hemorrhage after splenectomy and that one had only 7.8 grams of hemoglobin per 100 cc. of blood prior to the hemorrhage.

SUMMARY AND CONCLUSIONS

On the basis of observations on six animals the following conclusions are drawn:

1. General anesthesia of intact dogs with pentobarbital sodium administered intravenously causes a decrease of the concentration of hemoglobin.
2. Strenuous passive exercise after the administration of the anesthetic will not prevent the decrease of concentration of hemoglobin.
3. There is almost no decrease of concentration of hemoglobin when the splenectomized dog is anesthetized with pentobarbital sodium.
4. Acute hemorrhage (30 cc. per kgm. of body weight in 5 min.) of intact dogs anesthetized with pentobarbital sodium causes an increase of the concentration of hemoglobin followed by a slow decrease.
5. Acute hemorrhage of the splenectomized dog under pentobarbital sodium anesthesia causes a rapid decrease of concentration of hemoglobin.

REFERENCES

- (1) BOURNE, W., M. BRUGER AND N. B. DREYER. *Surg., Gynec. and Obstet.* **51**: 356, 1930.
- (2) ADOLPH, E. F., M. J. GERBASI AND M. J. LEPORE. *This Journal* **104**: 502, 1933.
- (3) ADOLPH, E. F. AND M. J. GERBASI. *This Journal* **106**: 35, 1933.
- (4) SEARLES, P. W. AND H. E. ESSEX. *Proc. Staff Meet., Mayo Clin.* **11**: 481, 1936.
- (5) ESSEX, H. E., S. F. SEELEY, G. M. HIGGINS AND F. C. MANN. *Proc. Soc. Exper. Biol. and Med.* **35**: 154, 1936.
- (6) HAUSNER, E., H. E. ESSEX AND F. C. MANN. *This Journal* **121**: 387, 1938.
- (7) SANFORD, A. H. AND C. SHEARD. *J. Lab. and Clin. Med.* **15**: 483, 1930.
- (8) PRICE, P. B., C. R. HANLON, W. P. LONGMIRE AND W. METCALF. *Bull. Johns Hopkins Hosp.* **69**: 327, 1941.
- (9) COOK, S. F. AND M. I. ROSE. *This Journal* **92**: 240, 1930.
- (10) BARBOUR, H. G. *Anesthesiology* **1**: 121, 1940.
- (11) LEHMAN, E. P. AND C. V. AMOLE. *Surgery* **4**: 44, 1938.

AUGMENTATION OF LEFT CORONARY INFLOW WITH ELEVATION OF LEFT VENTRICULAR PRESSURE AND OBSERVATIONS ON THE MECHANISM FOR INCREASED CORONARY INFLOW WITH INCREASED CARDIAC LOAD¹

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The coronary response to changes in the load put upon the right or left ventricle has been the subject of many investigations. In experiments with the isolated beating heart (1) or heart-lung preparation (1, 2, 3), elevation of right ventricular pressure by constriction of the pulmonary artery is reported to have reduced the blood supply to the myocardium of the right ventricle. Using the same types of preparation (1, 4, 5) and also with the heart beating *in situ* (6), the same and other investigators state that elevation of left ventricular pressure by aortic constriction (coronary perfusion pressure kept constant) caused a comparable reduction in blood flow to the myocardium of the left heart. However, in a recent communication from this laboratory (7), experiments were presented which demonstrated that progressive elevation of right ventricular pressure by constriction of the pulmonary artery, in the anesthetized open-chest dog, was accompanied by a considerable and sustained augmentation of right coronary inflow and a smaller but definite increase in left coronary inflow. The present report is a continuation of the investigation and embraces 1, the study of increased load upon the left heart versus left coronary inflow, and 2, consideration of the mechanisms responsible for the observed coronary inflow changes when the load upon either ventricle is increased.

Left coronary inflow versus elevation of left ventricular pressure. The animal preparation was similar to that used in the previous studies (7) and consisted of an anesthetized² dog under artificial respiration and the chest plate removed. After injection of anticoagulants³, the left common carotid artery was cannulated centrally and an appropriate rotameter (8) connected to the cannula. From the rotameter, blood was led to another cannula placed either in the left coronary artery or its circumflex branch. In the former instance, a special cannula was inserted into the aorta through a slit in the brachiocephalic artery and secured in the left coronary orifice by a tie around the vessel close to the aorta. Elevation of left ventricular pressure without elevation of central coronary pressure was effected in different experiments in two ways: 1. Both right and left coronary arteries were isolated at their respective origins and a cord passed beneath the aorta and another cord passed over the aorta, both being placed central to

¹ The expenses of this investigation were defrayed by a grant from the Commonwealth Fund.

² Sodium pentobarbital, 20 mgm./kgm.

³ Combination of heparin, 100 units/kgm. and pontamine fast pink, 150 mgm./kgm.

the coronary orifices. Lateral traction on the cords by means of a special retractor compressed the aorta in its anteroposterior diameter, thereby raising left ventricular pressure. 2. In some experiments, in which the left coronary artery was isolated and cannulated for flow measurement as above, the right coronary artery was cannulated 1 cm. or less from the aorta and independently supplied with blood from a side tube connected to the carotid cannula. In this instance, aortic constriction was accomplished by constriction of a "snare" loop of cord, placed around the ascending aorta peripheral to the coronary orifices. Central coronary perfusing pressure (aortic pressure) could be regulated in both cases by an adjustable clamp on the aorta near the diaphragm.

Elevation of left ventricular pressure by either method was consistently accompanied by a definite and sustained increase in left coronary inflow. The results graphically illustrated in figure 1A and B are representative of the changes which occurred in repeated determinations in each of six experiments. The increase in flow to the left heart which accompanies elevation of left ventricular pressure compares with the increased inflow to the right heart when right ventricular pressure is elevated (7).

Mechanism for increased coronary flow accompanying elevation of ventricular pressure. When the aorta or pulmonary artery is partially occluded, the resistance to systolic discharge is increased and the load under which the respective ventricles must operate also increases. If it is not overloaded, the heart is able to continue to function under an artificially increased load, at least within the limits of acute experiments. This is made possible, in part at least, by the fact that the blood supply to the heart is increased under these conditions, as shown here and in a previous communication (7). It is therefore not unreasonable to expect that an increased load upon either ventricle would demand a significant increase in coronary inflow if the work and metabolism are similarly increased. Accordingly, experiments were designed to estimate changes in the latter factors when the resistance load to either ventricle was increased.

The work of each ventricle was roughly estimated from the product of maximal ventricular pressure and cardiac input. The former was determined by a Gregg pressure manometer (9). Measurement of inflow to the heart was made with a large rotameter attached to a special cannula, designed by R. E. S. (cf. fig. 2) and inserted into both venae cavae⁴. Coronary oxygen utilization was estimated from the product of coronary flow and A-V O₂ difference. For the left ventricle, the A-V O₂ difference was determined from samples drawn from a side tube in the coronary artery cannula and from the great cardiac vein. For the right ventricle, the venous sample was taken from a cannulated anterior cardiac vein (10).

Typical data obtained with aortic and pulmonary artery constriction are presented in table 1, parts A and B. These show that 1, as ventricular pressure is raised, cardiac input is decreased proportionately less, thereby resulting in a net increase in work of the ventricle; 2, as the work is increased, the A-V O₂ dif-

⁴ The azygos and any other veins not included in the cannulation were tied off. In these experiments, venous blood returning from the coronary circuit was not included in the measurement of cardiac input.

ference is further increased, largely because of the reduction in venous O_2 content. The increased rate of coronary flow together with the increased A-V O_2 difference indicates a very considerable rise in O_2 utilization.

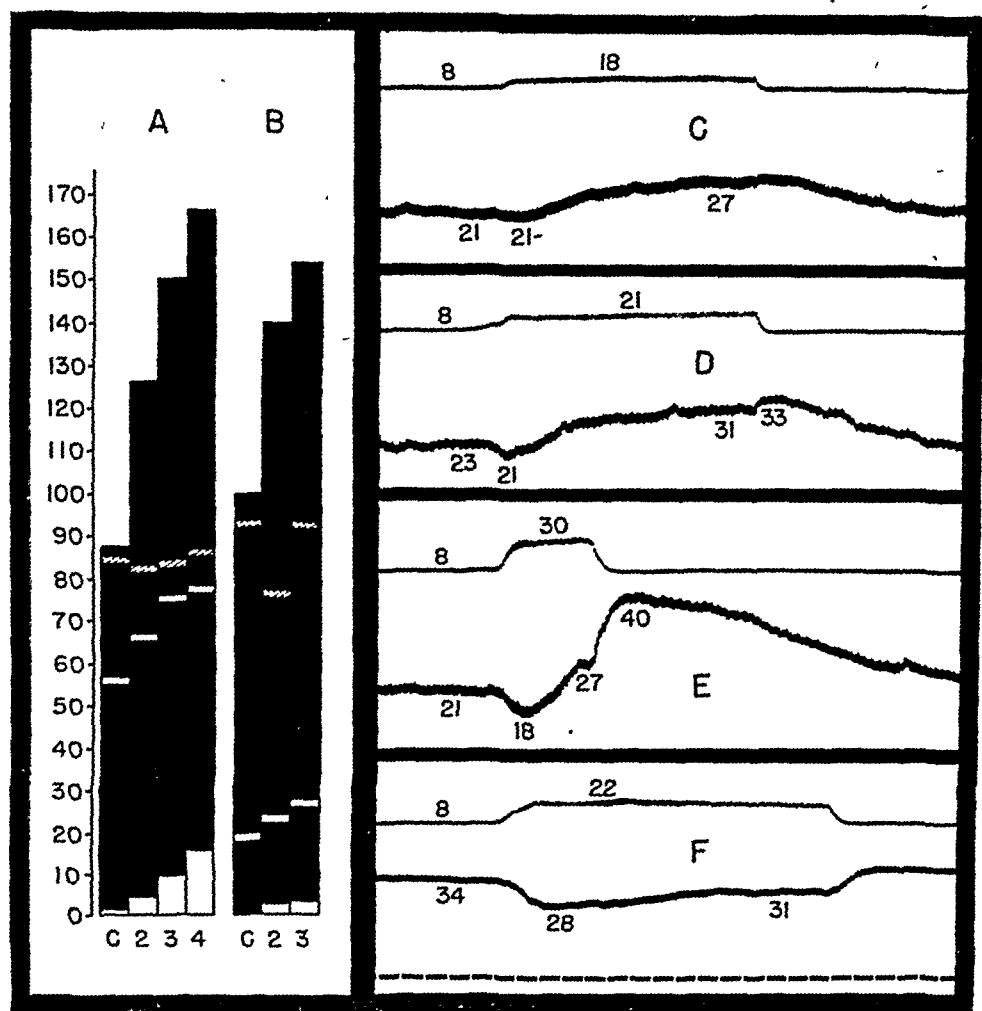


Fig. 1, parts A and B. Bar graphs showing the effect of augmentation of left ventricular pressure on coronary inflow; (part A, left coronary inflow; part B, left circumflex inflow). Solid black bars, systolic and diastolic left ventricular pressures; hatched segment within each bar, aortic (coronary perfusing) pressure; white segment within each bar, coronary inflow. Abscissa—C, control; numbers denote minutes' duration of aortic constriction. Common ordinate scale for all pressures in millimeters of mercury and flow in cubic centimeters per minute.

Parts C, D, E and F, photographs of original records showing the effects of elevation of right ventricular pressure on right coronary inflow. Upper curve in each segment, mean right ventricular pressure with values in millimeters of mercury indicated by numbers adjacent to curve. Lower curve, mean right coronary inflow with values in cubic centimeters per minute indicated by numbers adjacent to curve. See text for details and further description.

The preceding experiments have demonstrated that the increased O_2 utilization was related not only to the increase in rate of coronary blood flow but also to the more thorough extraction of O_2 from the blood. It was therefore of interest to

determine if the coronary flow increase would still occur if either the control O_2 content or rate or coronary flow were artificially elevated to supernormal levels.

Increased O_2 content. The preparation was the same as before except that O_2 was administered through the intratracheal tube. The control coronary A- VO_2 difference was thereby decreased and the O_2 content of the arterial blood increased almost to capacity. However, the rate of coronary inflow still increased with ventricular pressure elevation (cf. table, part C).

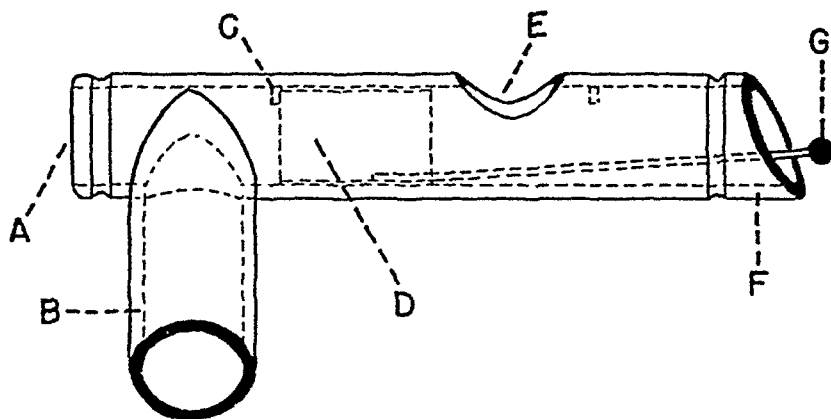


Fig. 2. Scale drawing ($1.2 \times$ actual size as seen from above) of special cannula used to measure cardiac input in dogs weighing from 8 to 12 kilo. Cannula is made from $\frac{1}{16}$ inch brass tubing with $\frac{1}{32}$ inch wall. Side tube (B) is soldered to, and communicates with, main tube. Thin-walled (0.006 inch) sleeve (D) fits snugly within main tube and rests against "stops" (C). A stiff brass wire connects sleeve with ball (G). Side opening (E) is drilled to same diameter as main tube. The cannula is inserted as follows: a short piece of rubber tubing is attached to side tube (B) and clamped. The superior vena cava is clamped cephalad and cut half way through about 1 cm. above its junction with the right atrium. The cannula end (F) is inserted through the short length of cava remaining, through the right atrium and on into the inferior vena cava where it is held in place by a tie around the groove at the end (F). The short segment of superior cava is secured with a tie around the main tube close to the junction with side tube (B). The superior portion of the superior cava is pulled over the cannula end (A), tied in place, and unclamped. Blood now enters the right atrium from both cavae through opening (E). A rotameter of proper capacity and sensitivity is attached to side tube (B) and its return tube attached to a large cannula tied in the tip of the right auricular appendage. Suction applied to the top of the rotameter replaces its contained air with blood. By manual traction through the thin-walled inferior cava, the ball (G) is pulled caudad in small increments until the sleeve (D) rests against other stop, in which position it shuts off the outflow through opening (E) and all blood passes through the rotameter before entering the heart.

Increased blood supply. For this experiment, the right coronary artery was perfused with blood under constant pressure, first at the prevailing aortic pressure and then at a much greater pressure both before and during pulmonary artery constriction. A rubber bulb with appropriate valves and an attached air chamber, inserted between the carotid cannula and rotameter, was intermittently compressed so as to give an essentially constant perfusing pressure as read on a mercury manometer. The data from such an experiment shown in table 1, part D, indicate that an increase in right coronary inflow still occurred during elevation of right ventricular pressure despite the fact that increasing the coro-

TABLE 1*

	(1) MEAN AORTIC BLOOD PRES- SURE	(2) RIGHT VEN- TRICU- LAR SYS- TOLIC PRES- SURE	(3) RIGHT CORO- NARY FLOW	(4) CARDIAC INPUT	(5) WORK TREND	COMMENTS	
			cc./min.	cc./min.	(2) × (4)		
A	88 85 86 80 79	18 43 45 56 63	10.0 13.2 13.2 15 15.8	680 628 655 590 604	12200 27000 29500 33000 38000	Control Pulmonary artery constricted 1 min. Pulmonary artery constricted 2 min. Pulmonary artery constricted 3 min. Pulmonary artery constricted 4.5 min.	Pulmonary artery constriction ver- sus work of right ventricle

				OXYGEN CONTENT		A-VO ₂ DIFF.		
				Right coro- nary artery	Ante- rior cardiac vein			
				vol. %	vol. %			
B	95-100 95-100 95-100 83 80	14 44 13 40 64	7.5 13.0 6.0 16.0 20.0	15.84 15.80 16.03 14.50 13.75	4.89 2.23 5.38 4.66 3.12	10.95 13.57 10.65 9.84 10.63	Control Pulmonary artery con- striction Recovery after release of pulmonary artery Control Pulmonary artery con- striction	Pulmonary artery constriction ver- sus A-VO ₂ dif- ference and O ₂ utilization
C	74 74 73	18 40 18	8.5 16.5 8.5	17.75 16.25 16.55	7.86 6.32 7.47	9.89 9.93 9.08	Control Pulmonary artery con- striction Recovery	Intratracheal oxy- gen administra- tion. Arterial O ₂ capacity
D	80 80 80 80	18 45 17 45	11.0 15.0 31.0 35.0	14.46 14.44 13.51 14.53	6.94 6.18 10.43 9.38	7.52 8.26 3.08 5.15	Control Pulmonary artery con- striction Control Pulmonary artery con- striction	Right coronary ar- tery perfused at aortic pressure Right coronary ar- tery perfused at 200 mm. Hg pressure

* We are indebted to M. Daus of this department for the blood gas analyses.

nary perfusion pressure from 80 to 200 mm. Hg almost trebled the control blood flow and O₂ supply.

Reflexes. Although intra- and extracardiac reflexes were considered as possible

contributing factors in augmenting coronary flow, no evidence has yet been obtained which would indicate the operation of such reflexes. Section of the vagi and of cardiac nerves from the sympathetic chain did not prevent the coronary flow increase with elevated ventricular pressure. Application of 2 per cent procaine to the pulmonary conus and pulmonary artery in the region of the constricting loop also failed to abolish the coronary flow response.

Origin of vasomotor response. The experiments presented here and in a previous communication (7) have shown that increasing the load upon either ventricle by constriction of the pulmonary artery or aorta (central to the coronary orifices) increases the work, the metabolism and the coronary flow of the respective ventricle. The mechanism responsible for dilatation of the coronary vessels cannot be identified with certainty. However, since the flow response rather closely parallels the changes in work, it is not improbable that the associated changes in metabolism are capable of effecting vasomotor regulation of the blood supply to the involved ventricle. Two possible mechanisms can be advanced. The coronary vasodilatation results from 1, an increased local production and release of metabolites, and/or 2, the creation of local relative anoxia, caused by a disproportion between the increased rate of O_2 utilization and the existing coronary blood flow; partial (but not complete) compensation is accomplished by vasodilatation and increased blood flow.

Effect of extravascular compression. Regardless of the mechanism by which coronary inflow is increased, elevation of right or left ventricular pressure can also be shown to have a flow reducing effect which operates in antagonism to the flow promoting mechanisms. An example will serve to demonstrate these separate effects. For the following experiment the preparation was as described earlier with the rubber bulb apparatus for supplying blood at constant perfusing pressure. To obtain an accurate picture of the temporal relation of events, right coronary inflow was recorded continuously and optically by the recording rotameter described elsewhere (11, 12). Figure 1C is a simultaneous recording of mean right intraventricular pressure and right coronary inflow. In this instance, partial occlusion of the pulmonary artery slightly more than doubled the mean pressure in the right ventricle. After a short lag, right coronary flow progressively rose until the pulmonary artery was released, after which the rate of coronary inflow gradually returned to the control level. The procedure was repeated (fig. 1D); right ventricular pressure rose somewhat more and the coronary inflow underwent a slight but temporary drop before rising gradually. In addition, release of the pulmonary artery was accompanied by a temporary but further increase in coronary flow before returning to the control level. These records are typical of the changes in flow observed in many experiments and described in a previous communication (7). In figure 1E are shown the results obtained with a more severe and abrupt constriction of the pulmonary artery. Here again, the coronary inflow increased (from 21 to 27 cc/min.) but only after a 13 second period of decreased flow. Coincident with the release of the pulmonary artery constriction, the flow abruptly rose to a very high level and thereafter gradually returned to the control. These transient but abrupt changes in flow coincident with the onset of elevation and reduction in right

ventricular pressure are regarded as evidence of a temporary separation of the influence of change in extravascular compression upon coronary flow. This conclusion is made with reasonable justification since the increase in mechanical compression of the coronary vessels occurs simultaneously with the increase in intraventricular tension while the slower physiological metabolic and vasomotor responses must necessarily lag somewhat behind their respective exciting causes. Hence, the temporary decrease in flow can be attributed to the dominant influence of augmented mechanical compression. The subsequent appearance of an increased flow observed shortly thereafter indicates that the effect of coronary dilatation has exceeded the flow reducing effect of the increased extravascular compression. The immediate but transient increase in flow following the abrupt release of the pulmonary artery can be regarded as a rough index of the extent to which the flow had previously been retarded by extravascular compression.

"Abnormal" responses. The changes in coronary flow illustrated in figures 1C, D, E demonstrate what is believed to be the "normal" relationship between increased ventricular load and coronary flow. Under these conditions, the net physiological response is directed toward augmenting the coronary blood supply in spite of the flow limiting effect of increased extravascular compression. However, if the animal experiment is intentionally prolonged (for several hours), the flow response is reversed and the effect of a sustained and unalterably dominant influence of extravascular compression can be demonstrated. This response is shown in figure 1F, a record made from the same animal preparation about one hour after that in figure 1E. During that interval, the dog's blood pressure fell somewhat (from 90 to 80 mm. Hg mean pressure) but no saline infusions or stimulant drugs were given. The right coronary flow response to pulmonary artery constriction is now a sustained decrease which returns to the control level upon release of the pulmonary artery. This response could be obtained repeatedly and in no instance did the coronary flow exceed or even reach the control value. In this late stage of the experiment it is obvious that the effect of increased extravascular compression in mechanically reducing coronary flow is left almost entirely unopposed by any concomitant dilatation of the coronary bed. In any event, the inability of the heart to increase or even maintain its blood supply in the presence of an augmented load indicates an almost complete lack of "reserve". The heart must be regarded as having suffered some change or loss in its physiological mechanisms, which event makes impossible the observation of those occurrences previously existent under more nearly "normal" physiological conditions.

This latter observation is consonant with that of other investigators, using heart-lung or isolated heart preparations, who report the consistent finding that flow through perfused arteries decreases with elevated intraventricular pressure (1, 2, 3, 4, 5). The same flow response is reported with open-chest but otherwise "intact" dogs in which the hearts underwent rather severe manipulation as the result of the methods employed (6). In all instances (including the later stages of the experiment described above), there is sufficient cause to believe that the hearts were probably incapable of any other than an "abnormal" response. This response, a persistent decrease in coronary flow, must be regarded as the *normal*

response for the existing state of the heart and as such merits interpretation only in relation to the preparation and the conditions under which it was obtained. While it is of some physiological interest to know under what circumstances a persistent decrease in coronary flow may accompany an increased cardiac load, the procedures or conditions necessary for its demonstration create a preparation so far removed from normal that the finding would appear to have little if any significance in relation to normal cardiac function. On the other hand, it is not to be claimed that short experiments with anesthetized open-chest dogs necessarily reveal the normal occurrences in the unanesthetized dog. However, this preparation, together with adequate methods and instruments, appears to offer a much more reliable approach toward the attainment of that end.

SUMMARY

Left coronary inflow is found to increase significantly in the anesthetized open-chest dog, when the load upon the left ventricle is increased by constriction of the aorta (central to the coronary orifices). This flow response is similar to that obtained previously in the right coronary artery in the presence of pulmonary artery constriction.

Measurement of cardiac input and oxygen consumption in the presence of an augmented load on either right or left ventricle has demonstrated an increase in the work and metabolism of the respective ventricle. In consideration of the increased coronary inflow observed, the two ventricles have at their disposal a compensatory means by which their blood supply can, at least in part, be adjusted to their work and metabolic requirements. It is suggested that the coronary dilatation arises from an increased local production of metabolites and/or the creation of local relative anoxia due to increased O_2 utilization.

If the experiments are intentionally prolonged (for several hours) and the coronary artery is perfused at constant pressure, the coronary flow response ultimately becomes a sustained decrease. As observed under these conditions, and by others using heart-lung and isolated heart preparations, the decrease in coronary inflow is regarded as an "abnormal" response to an increased cardiac load.

REFERENCES

- (1) KATZ, L. N., K. JOCHIM AND A. BOHNING. *This Journal* **122**: 36, 1938.
- (2) MOE, G. K. AND M. B. VISSCHER. *Blood, heart and circulation*. Pub. no. 13, A.A.A.S., 1939, p. 100.
- (3) VISSCHER, M. B. *J. A. M. A.* **113**: 987, 1939.
- (4) ANREP, G. V. AND H. HAUSLER. *J. Physiol.* **65**: 358, 1928.
- (5) DAVIS, J. C. *This Journal* **123**: 50, 1938.
- (6) GREEN, H. D. AND D. E. GREGG. *This Journal* **130**: 126, 1940.
- (7) GREGG, D. E., W. H. PRITCHARD, R. E. SHIPLEY AND J. T. WEARN. *This Journal* **139**: 726, 1943.
- (8) GREGG, D. E., R. E. SHIPLEY, R. W. ECKSTEIN, A. ROTTA AND J. T. WEARN. *Proc. Soc. Exper. Biol. and Med.* **49**: 267, 1942.
- (9) GREGG, D. E. AND D. DEWALD. *This Journal* **124**: 435, 1938.
- (10) GREGG, D. E., R. E. SHIPLEY AND G. BIDDER. *This Journal* **130**: 732, 1943.
- (11) SHIPLEY, R. E. AND E. C. CRITTENDEN, JR. *Proc. Soc. Exper. Biol. and Med.*, June, 1944.
- (12) CRITTENDEN, E. C., JR. AND R. E. SHIPLEY. *Rev. Scientific Instruments*, in press, 1944.

RESPIRATORY EFFECTS ON THE FILLING OF THE VENTRICLES DURING A PROLONGED DIASTOLE

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In an earlier paper (Boyd and Patras, 1941) we described some respiratory effects on cardiac filling and output at ordinary rates of beat. A cardiometer was used, with the chest closed and the animal breathing naturally. Under such conditions, if diastole is prolonged by vagal stimulation, and the procedure repeated at random intervals, the filling curves registered vary markedly in form. Each variation, however, is characteristic of some part of the respiratory cycle, and presumably is determined by a respiratory change of effective venous pressure. The most conspicuous departures from the conventional type of curve appear in the later stages of diastole, during the period of so-called diastasis. The present report is concerned with these respiratory effects on the course of ventricular filling.

METHODS. Large dogs (14 to 25 kgm.) were used. The anesthetic was either barbital-sodium (0.3 gram per kgm., given intraperitoneally) or the combination of morphine and barbital recommended by Wiggers (1942, p. 79).

When a cardiometer is properly adjusted over the ventricles, the effective venous pressure is the blood pressure in the atria measured against the air pressure in the cardiometer. If normal relations are to be maintained, the pressure acting on the ventricles should always be the same as that applied to the surfaces of the atria and veins from which the blood must enter. After the chest is closed and subatmospheric pressure re-established within it, the ventricles in the cardiometer may be subjected to abnormal pressures from two sources. First, the recording device attached to the cardiometer may be so resistant that the ventricles cannot change their volume without considerable compression or rarefaction of the air around them. Second, the pressure beyond the recording device, against which it must move, may be different from the intrathoracic pressure. To avoid the second difficulty we have used a recording tambour inclosed on both sides (Boyd and Patras, 1941). The air space on one side is connected to the cardiometer, that on the other to the thoracic cavity of the animal. Volume changes of the ventricles are balanced by equal displacements between the opposite side of the tambour and a small volume of air left free in the thorax. Thus pressure on the ventricles is always equal to the varying intrathoracic pressure, except for differences arising from the resistance of the recording system.

The resistance of the recording system presents a more difficult problem, recognized by earlier workers but never satisfactorily met. In the various types of apparatus so far available, a frequency adequate for recording volume over the entire cycle can be secured only by translating volume changes into considerable pressure changes (Wiggers and Katz, 1922). The tambour described by Wiggers

and Katz has a satisfactory frequency, but is so resistant that a volume change of 30 cc. produces in the cardiometer a pressure change of 31 mm. of water. Such an instrument must keep the ventricles, over most of each cycle, under a pressure either greater or less than that acting on the vessels upstream, and diastolic filling would always proceed against a steadily increasing external resistance added to the natural resistance of the ventricles themselves. It is difficult to see how the course of filling could remain unaffected thereby. It is true, as Wiggers and Katz point out, that the heart beat normally causes changes of pressure in the thoracic cavity, but this seems to us irrelevant. Changes of intrathoracic pressure occurring normally are transmitted, to act, so far as is known, on all the intrathoracic vessels alike. The pressure changes produced in a cardiometer by instrumental resistance are applied to the surfaces of the ventricles only, creating local pressure gradients which do not normally exist.

We are convinced, therefore, that a frequency adequate for recording the entire volume curve can be attained with tambours only at the price of distorting pressure relations. Since the degree of distortion will vary according to the magnitude of the volume changes, it might be held within tolerable limits by using small animals. In our experience, however, the respiration of small dogs always is seriously embarrassed by the presence in the closed chest of a cardiometer, no matter how carefully it be adapted to the size of the heart. Large dogs usually show little dyspnea or other visible disturbance, but the combined stroke output from both ventricles, after a prolonged diastole, may be as much as 60 cc. In a tambour of adequate frequency this would entail a pressure change of approximately 6 cm. of water. Since the effective venous pressure normally amounts only to 5 or 6 cm., the error introduced by such a tambour, for the present study at least, would appear to outweigh any advantage gained.

Fortunately a more sensitive instrument can follow the gradual volume changes over the greater part of diastole, even though other parts of the cycle may not be recorded with entire accuracy. We have used a double tambour similar in dimensions to that described in our earlier paper. The membrane, of light rubber dam under low tension, allows a volume change of 80 cc. (40 cc. in each direction from its mid position) with a total pressure change not exceeding 8 mm. of water. Limitation of pressure variations to this low range has the incidental advantage of minimizing the tendency toward leakage of air at the ring of contact between the heart and the membrane of the cardiometer. This makes it relatively easy to avoid undesirable constriction at the A-V groove. Frictional resistance to air movement was kept low by using short connecting tubes of 10 mm. bore.

The tambour was modified in construction to permit recording on smoked paper. Since in operation the air pressure on both sides of its membrane is subatmospheric, while the free end of its lever must move in outside air, the ordinary type of fulcrum obviously cannot be used. In the wall of the upper chamber (fig. 1A) a vertical slit window (W), 2.5 mm. wide, is covered with thin, taut rubber dam. A light hollow lever of glass, 16 to 18 cm. long, is made by drawing out a thin-walled test tube to a diameter of about 1.5 mm., and sealed at the ends. This lever is thrust through a needle puncture in the rubber dam,

centered over the window, and its inner end engaged in a hole through the flat vertical shaft *S*. The fulcrum of the lever is the point at which it pierces the rubber dam, the puncture being sealed around it with rubber cement. The subatmospheric pressure in the tambour prevents the membrane from ever bulging outward. Inward bulging is prevented by its tension and the narrowness

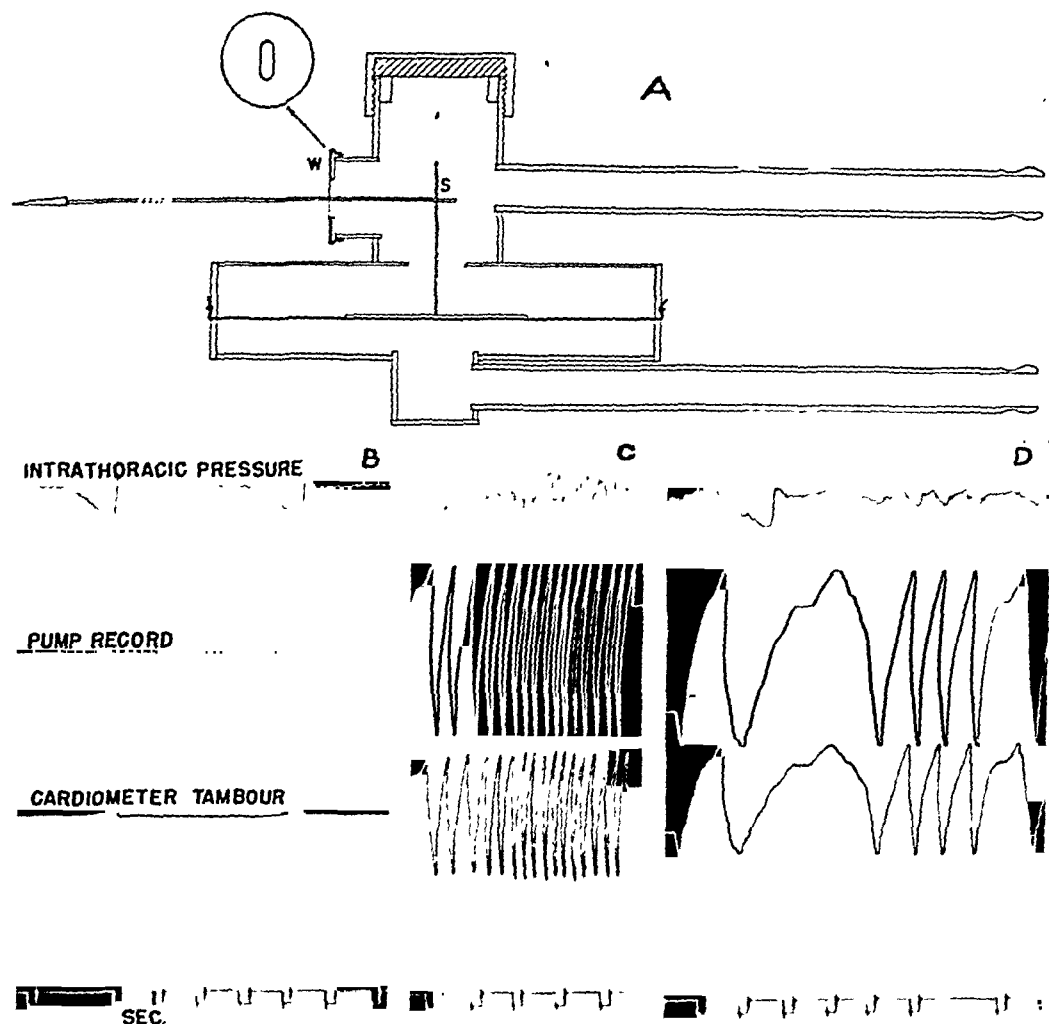


Fig. 1. Above, sectional diagram of cardiometer tambour.

Below, records of intrathoracic pressure, movements of a pump piston and movements of the tambour. On the left the pump is stationary and respiratory excursions of the tambour are shown. Center and right, tambour recording volume changes from pump. Stroke volume of pump, 47.8 cc.

Further description in text.

of the slit behind it. To record ventricular filling by an upward stroke of the lever, the upper chamber of the tambour is connected to the cardiometer.

The recording system was tested by connecting the upper chamber to the outlet of a cylindrical pump driven at irregularly varying speeds. The lower chamber was connected to the thoracic cavity of a dog, with a closed pneumothorax sufficient to allow displacement of air by the pump to be compensated by

equal movements into and out of the thorax. Excursions of the pump piston and of the tambour membrane were recorded simultaneously, with levers of the same length to equalize their arcs. It will be noted (fig. 1, C) that the tambour shows a small fling at pump speeds exceeding 3 per second, but that at lower speeds the volume changes are recorded without appreciable error. The apparatus thus seems capable not only of recording volume during a prolonged diastole, but also of registering any gross variations of stroke output.

The membrane is slightly displaced by the respiration, independently of the heart. This effect is due to alternate expansion and compression of the air on the cardiometer side of the membrane as the counter pressure on the opposite side changes. The record of ventricular volume must therefore be referred to a base line rising with inspiration and falling with expiration. The respiratory displacement may be recorded in uncomplicated form by connecting the lower chamber of the tambour in the usual manner to the thorax, and the upper chamber to a flask with a closed air space (figs. 1B, 2). From the data so obtained the shifting base line may be constructed approximately and drawn into the record (fig. 2, A to D).

The respiratory displacement is directly proportional to the pressure change and to the total volume of air held on the cardiometer side. This volume cannot be precisely determined in practice. The capacity of the upper chamber and its connecting tubes, when the membrane is plane, is about 195 cc. To this may be added the known capacity of the cardiometer, but a deduction must be made for the volume occupied by the ventricles. Their volume is unknown and will inevitably change if the pressure upon them is altered in any attempt at calibration. An arbitrary estimate may be used, however, without introducing serious error into the result. Since the pressure in the system is never far below atmospheric, a pressure change of 1 mm. Hg changes volume only by about $1/750$. Hence the respiratory displacement is always small. In calculating the air volume to be used in controls we have made a deduction of 130 to 180 cc. to allow for the mean volume of the beating ventricles.

Arterial pressure was recorded by a mercury manometer, from the right carotid artery. Venous pressure was not continuously recorded, but direct readings were made from time to time. A sound was passed through the jugular vein into the right atrium. Externally it was connected through a Y-tube to a vertical manometer tube of 4 mm. bore, and to a reservoir containing saline solution with 0.2 per cent of chlorazol fast pink added. Into this reservoir a fine spray of mercury was delivered continuously from a second reservoir above, displacing the saline and feeding it slowly into the circulation. Such an arrangement, with a different anticoagulant, was used by Trendelenburg (1924). A flow of about 20 cc. per hour was found sufficient to prevent clotting.

Venous pressure was observed before and during adjustment of the cardiometer, and the membrane of the latter refitted if it caused a rise of more than 1 cm. After the chest was closed, the upper end of the venous pressure manometer tube was connected to the thoracic cavity, through a side outlet on the line leading to the cardiometer tambour. Direct readings of venous against

intrathoracic pressure could thus be made at any time, and referred later to a zero level determined at the end of the experiment. The animal was finally killed by bleeding from an artery, the chest opened and the atrial wall above the A-V orifice cut away with the sound still in place. When the liquid in the manometer had fallen to a steady level, the reading at that point was taken as the zero for venous pressure.

When such a manometer, previously open to the outside air, is connected to the thoracic cavity, the mean level of liquid naturally rises, and the respiratory fluctuations are reversed in direction. The pressure rises with inspiration, but readings made at that time are probably inaccurate because of the inertia of the system. Readings given below were all made at the steady level reached during the expiratory pause.

The right vagus nerve was sectioned, and tetanic stimuli applied for brief periods to its peripheral stump.

RESULTS. Figure 2 illustrates the main variations appearing in the filling curve at different times in the respiratory cycle. The upper section shows four original records from one experiment, each made at a time when the effective venous pressure, at the expiratory level, was 52 mm. of saline. The lower section shows the same curves redrawn, to eliminate horizontal and vertical distortion caused respectively by the arc of the lever and by the inspiratory shift of base line. In A, diastole begins and is completed during the expiratory pause. This filling curve conforms to the conventional type, and is redrawn paired with each of the others for comparison. The remaining three all show the accelerating effect of inspiration on the rate of filling. The form of the curve, however, depends on the time relations between the beginning of diastole and the onset of inspiration. B, beginning late in the expiratory pause, is identical with A until diastasis is well under way. Inspiration then starts and brings a secondary acceleration of inflow. In C inspiration begins before diastasis has set in, and the effect is simply to prolong the initial period of rapid inflow with a relatively steep gradient throughout the later stages. D begins when inspiration has reached an advanced point. This curve is steeper than A even during the initial period of rapid inflow of the latter. D shows also the effect of expiration occurring while diastole is still in progress. The volume curve is abruptly flattened and a reverse flow is registered just before atrial systole.

At subnormal venous pressures the respiratory effects on ventricular filling are even more obvious (fig. 3, B). At higher mean levels of venous pressure they are relatively smaller but still noticeable (fig. 3, A). When several vagal beats are recorded in succession, conditions are complicated by the gradual rise of venous pressure and by changes in the rate and depth of respiration. Variations in the filling curve appear, however, throughout the series (fig. 3, C).

The residual volume of blood left in the ventricles at the end of systole is almost invariably greater during inspiration than during expiration or the ensuing pause. On the original records this difference in systolic levels is exaggerated by the inspiratory rise of base line, but it is still evident when due correction is made. It has already been noted that at ordinary heart rates,

the maximum stroke output occurs when systole coincides in time with expiration (Boyd and Patras, 1941; Shuler, Ensor, Gunning, Moss and Johnson, 1942). We make no attempt to analyze the systolic volume curve, since the changes are too rapid to be followed in detail by the technique here used.

In the examples shown, it happens that the arterial pressure was relatively low. We have made comparable observations, however, on 18 dogs, at varying

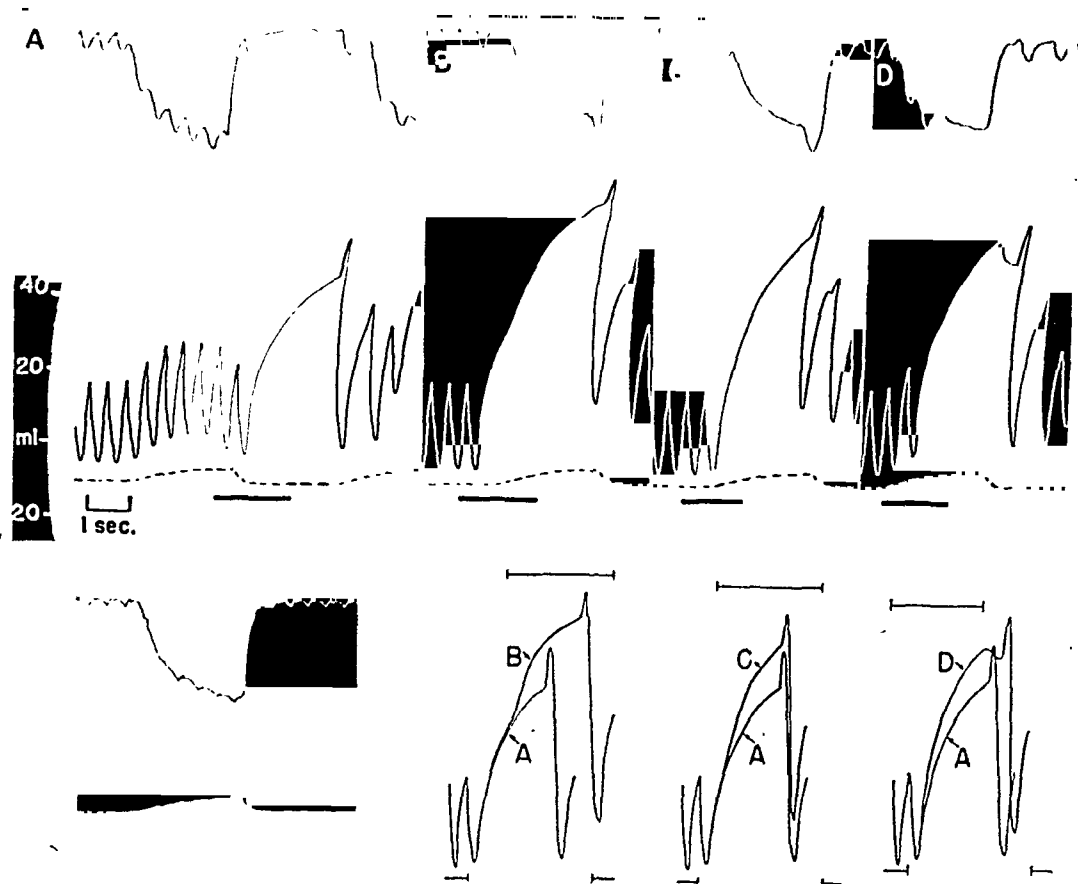


Fig. 2. Above, records of intrathoracic pressure and ventricular volume from a dog of 17.4 kgm. Both vagi sectioned. Arterial pressure 90 mm. Hg, effective venous pressure 52 mm. saline. White bars indicate vagal stimulation, the dotted white lines the respiratory shifts of base level for the volume record.

Below, left, movements of the cardiometer tambour caused by respiration alone. Right, records from above reconstructed and redrawn, B, C and D being each superimposed on A for comparison. Lines at the bottom of each drawing indicate the beginning and end of inspiration for record A. Lines at the top show the limits of the inspiration for each of the other records.

Further description in text.

levels of arterial pressure, and have always found the respiratory effects on filling described above. We do not believe, therefore, that the behavior can be attributed to an abnormal condition of the heart.

It may be noted that if the recording tambour is arranged to move against outside air, the ventricles must be filled against a constant atmospheric pressure. When such an arrangement is used, with experimental conditions other-

wise unchanged, the effects described above are reversed in direction. Ventricular filling is accelerated by expiration and retarded by inspiration (cf. Boyd and Patras, 1941).

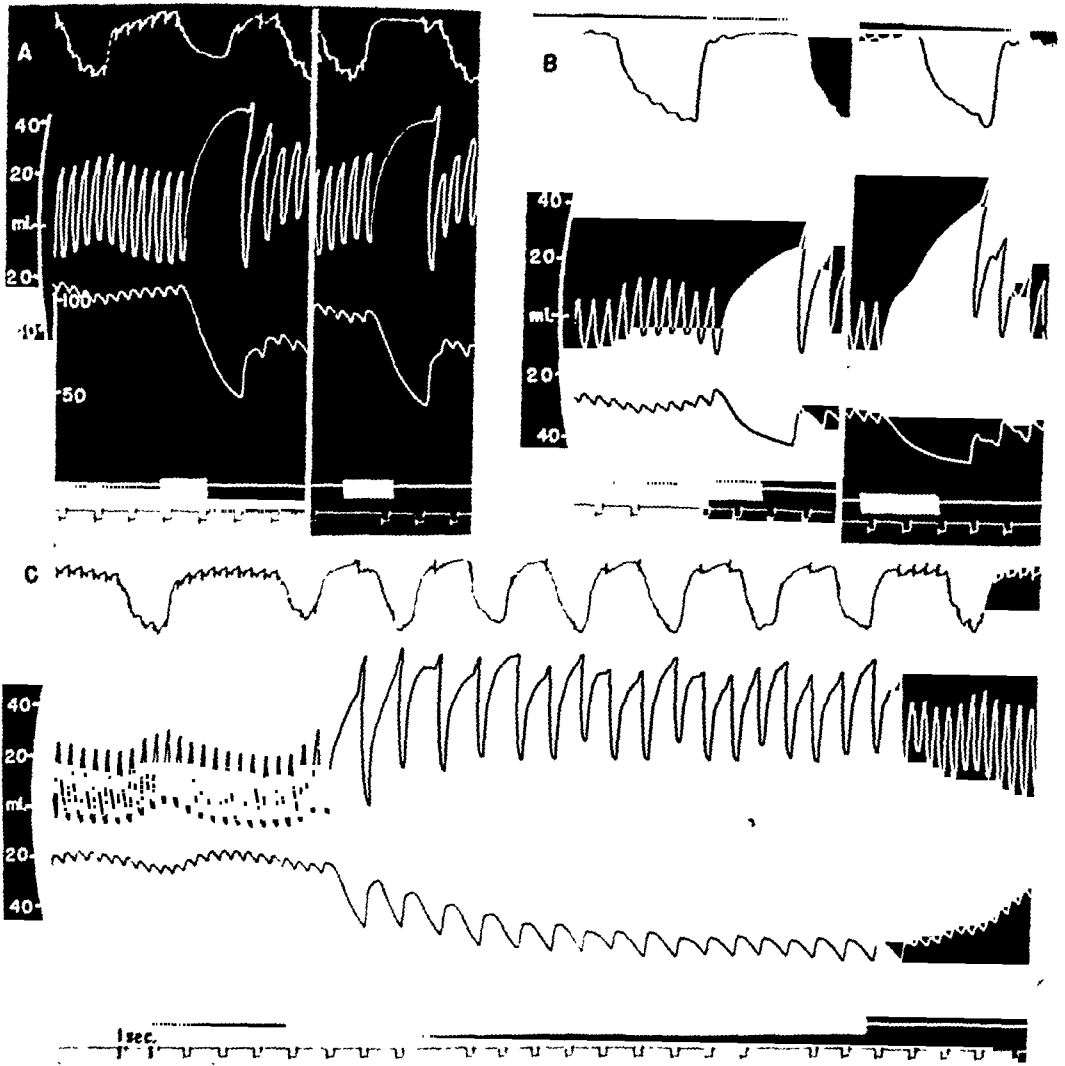


Fig. 3. A, intrathoracic pressure, ventricular volume and arterial pressure records from a dog of 20.8 kgm. Effective venous pressure 93 mm. saline. Vagal stimulation at signals.

B. Similar records from a dog of 17.4 kgm., effective venous pressure reduced by hemorrhage to 39 mm. saline.

C. Record of a series of vagal beats. Same animal as in A. Effective venous pressure at beginning of vagal stimulation was 72 mm. saline, at end 185 mm.

DISCUSSION. Aside from disturbances entailed by anesthesia and by surgical preparation, our experimental conditions obviously were abnormal in two important respects. These are the prolonged period allowed for ventricular filling, and the removal of whatever restraint the pericardium normally may exert. It is probable that in the intact animal respiratory variations of ventricular filling are comparatively small. At ordinary heart rates the filling period is brief, and it may be further shortened during inspiration by a respiratory

arrhythmia. The resistance offered by the pericardium to ventricular distention appears to be determined largely by the pull on its diaphragmatic attachment (Wilson and Meek, 1927). If so, the resistance might be expected to increase during inspiration. These factors would tend to offset the inspiratory rise of filling pressure.

The observations here reported seem, however, to have some bearing on the mechanism of ventricular filling. Y. Henderson (1909; see also Henderson and Barringer, 1913) held that the filling curve, except at abnormally low levels of venous pressure, is determined simply by the rate of relaxation of the ventricles. This view implies that the length of the muscle fibers at any given moment is fixed by their inherent properties, and cannot be altered by the ordinary variations of distending pressure. Wiggers and Katz (1922), however, showed that if the mean level of venous pressure be experimentally raised, the filling curve becomes steeper throughout its course; and that this effect appears over a wide range of venous pressures. It seems to follow that diastasis cannot be merely a continuing slow relaxation of the ventricles, but is a state in which the rate of inflow at any given moment depends on two opposing forces, filling pressure and the increasing tension in the passively stretched muscle. These forces are nearly balanced, and the filling curve is a graph of the rate at which they are approaching equilibrium. The smooth parabolic form of this curve, as shown on conventional diagrams, must then be characteristic only of stabilized conditions, in which there is a steady venous pressure throughout the cardiac cycle except for those changes which are directly attributable to mechanical events in the heart itself. Deviations from the familiar type of curve might be expected whenever the balance of forces is disturbed by any agency acting from outside the heart. Abrupt changes in its gradient always appear at atrial systole and diastole. There is no obvious reason why similar effects might not be produced earlier on the curve by any change of venous pressure imposed from upstream. Changes of effective venous pressure take place frequently under normal conditions, and recur regularly in the respiratory cycle. It has been shown here that these changes not only influence the general slope of the filling curve, but also may produce sudden alterations of gradient and even reversals of direction.

SUMMARY

1. Ventricular volume was recorded by means of a cardiometer in the closed chest, the recording system being so arranged that external pressure on the ventricles followed the normal respiratory variations of intrathoracic pressure. Diastole was prolonged by vagal stimulation.

2. Under these conditions, ventricular filling at any stage of diastole is accelerated by inspiration and retarded by expiration. If either inspiration or expiration occurs while filling is in progress, the gradient of the filling curve is altered thereby. The volume curves thus vary considerably in form. Such variations are particularly prominent during the stage of diastasis.

3. The effects described become less conspicuous as the mean level of venous

pressure is raised, but are still demonstrable when venous exceeds intrathoracic pressure by 90 mm. of water.

REFERENCES

- BOYD, T. E. AND M. C. PATRAS. This Journal 134: 74, 1941.
HENDERSON, Y. This Journal 23: 345, 1909.
HENDERSON, Y. AND T. B. BARRINGER. This Journal 31: 352, 1913.
SHULER, R. H., C. ENSOR, R. E. GUNNING, W. G. MOSS AND V. JOHNSON. This Journal 137: 620, 1942.
TRENDELENBURG, P. Pflüger's Arch. 203: 413, 1924.
WIGGERS, C. J. Physiol. Rev. 22: 74, 1942.
WIGGERS, C. J. AND L. N. KATZ. This Journal 58: 439, 1922.
WILSON, J. A. AND W. J. MEEK. This Journal 82: 34, 1927.

TISSUE ELECTROLYTE AT LOW ATMOSPHERIC PRESSURES

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The following paper presents analyses of cats' brains, muscles and hearts for electrolyte after exposure to low atmospheric pressure both with and without the addition of carbon dioxide.

EXPERIMENTAL PROCEDURES. The chamber¹ has a capacity of about 270 liters. A continuously operating pump reduces the pressure and the degree of reduction of pressure is controlled by valves regulating the ventilation into the tank at this point. Continuous records of the pressure are kept. In the experiments involving addition of carbon dioxide, the intake of carbon dioxide was regulated through a flow gauge while in the other experiments the inlet received only room air. The composition of the air in the chamber was determined by frequent analyses for carbon dioxide with a Haldane apparatus and the intake of carbon dioxide adjusted so as to give approximately the desired partial pressure.

Two runs of 23.5 hours with 3 cats each were obtained without addition of carbon dioxide. The pressures varied less than 15 mm. Hg from 355 mm. This would give an oxygen tension of about 71 mm. Hg. Two runs of 24 and 23 hours with two cats each were obtained with the addition of carbon dioxide. Except during the first three hours, the pressures were almost as constant, being within 20 mm. of 370. During the last 12 hours in the chamber, the average carbon dioxide pressures were 32 and 33 mm. Hg with a variation of 5 mm. from this figures. This would produce an oxygen tension of about 67 mm. Hg.

On removal from the chamber, the cats were anesthetized with 40 mgm. of nembutal per kilogram and killed by bleeding from the femoral artery within 20 minutes. Tissues and blood were analysed as in previous studies (1). Blood pH was determined at 38 degrees with a glass electrode. In the tables previous analyses of cat tissues are used, but only two determinations of pH in controls were available using the same technique in handling the cats as well as in estimating the pH. These gave the identical value of 7.25.

In the brain the analyses are expressed per 12 grams of total nitrogen. This amount of nitrogen is the average per 100 grams of fat free solids in normal cats. It was felt advisable to express the values in this manner since the data show variations due to lack of uniformity in the dried samples. This lack of uniformity is chiefly dependent on the fact that part of the fat was melted during the drying and could not be equally dispersed through the ground sample. Hence

¹ The author is indebted to the Department of Physiology of Yale University for the use of a small chamber. In particular he is grateful to L. C. Nims and R. W. Clarke who assisted in managing the controls of this chamber.

the fat determinations involve the greatest errors. The results, therefore, are more constant if calculated per 12 grams of nitrogen than per 100 grams of fat free solids. The brain analyses may be considered essentially the same as if calculated per 100 grams of fat free solids. All other tissue analyses are calculated per 100 grams of fat free solids. The serum analyses are expressed in the usual terms per volume of serum.

RESULTS. As shown in table 1, cats normally have a higher concentration of serum chloride than most mammals. Chloride concentrations do not change during 24 hours at low atmospheric pressure. The normal high chloride of cats

TABLE 1
Concentrations per liter of serum

GROUP	NUMBER	H ₂ O	pH	pCO ₂	HCO ₃	Cl	Na	K
		<i>gram</i>		<i>mm.</i>	<i>mM</i>	<i>mM</i>	<i>mM</i>	<i>mM</i>
Control.....	34	932	7.25	46	20.4	119.8	149.5	5.7
S.D.....		1.0			3.6	2.5	2.5	0.6
355.....	6	930	7.14	30	12.6	119	147.5	6.3
S.D.....		3.8	0.07	2.5	1.1	3.0	0.9	0.5
370 + CO ₂	4	928	7.14	57	19.0	120	152.8	5.2
S.D.....		2.5	0.07	0.1	0.9	3.5	1.2	1.3

TABLE 2
*Brain water and electrolyte**

GROUP	NUMBER	H ₂ O	N	Cl	Na	K	P	Fat
		<i>gram</i>	<i>gram</i>	<i>mM</i>	<i>mM</i>	<i>mM</i>	<i>mM</i>	<i>gram</i>
350.....	6	533	12.0	24.9	36.6	64.8	68.8	50
S.D.....		7.5		2.5	1.0	1.2	0.6	6
370 + CO ₂	4	508	12.0	23.4	35.2	63.0	70.0	45
S.D.....		21		2.4	1.1	1.2	1.2	2
Control*.....	11	533	12.0	25.0	37.3	67.0		46
S.D.....		12	0.6	2.4	2.7	5.4		5
Control.....	1	531	12.0	23.4	35.9	63.8	66.8	

* Controls expressed per 100 grams of fat-free solids.

All other analyses per 12 grams of nitrogen.

The single control was analysed along with an experimental group.

is reflected in a normal high sodium concentration when compared to other animals. On exposure to low atmospheric pressure, there is significant decrease in serum sodium when no carbon dioxide is added and a significant increase in serum sodium when carbon dioxide is added. The decrease in serum sodium at low atmospheric pressure is accompanied by a decrease in serum bicarbonate. However, there is no decrease in serum bicarbonate when carbon dioxide is added to air at low atmospheric pressure. Quantitatively the changes in serum sodium are not sufficient to account for all the changes in serum bicarbonate.

The determinations of pH of the blood cannot be considered representative of

conditions in the chamber since they are altered by the effects of the anesthesia on breathing and by removal of the cats from the chamber. The results of determinations of bicarbonate and pH suggest that in cats adjustment to the alkalosis of overbreathing is obtained not by increase in serum chloride but by decrease in serum sodium. In any case serum bicarbonate decreases at low atmospheric pressure and this change is prevented by addition of carbon dioxide to air having similar oxygen tensions.

The tissue analyses are represented in tables 2, 3 and 4.

In the brain there are no certain changes demonstrated. Both with and without carbon dioxide, the brain potassium is somewhat low (standard deviation of the difference about twice the difference, indicating probable significance).

None of the differences are significant in skeletal and heart muscle.

TABLE 3

Muscle water and electrolyte concentration per 100 grams of fat-free solids

GROUP	NUMBER	H ₂ O	N	Cl	Na	K	P
		<i>gram</i>	<i>gram</i>	<i>mM</i>	<i>mM</i>	<i>mM</i>	<i>mM</i>
Control.....	24	345.0	15.3	5.9	8.0	47.4	33.5
S.D.....		18	0.17	1.1	1.1	2.3	1.7
350.....	3	353	14.6	4.7	7.4	47.7	33.0
370 + CO ₂	4	345	15.1	6.2	7.9	46.1	31.5

TABLE 4

Heart water and electrolyte concentration per 100 grams fat-free solids

GROUP	NUMBER	H ₂ O	N	Cl	Na	K	P
		<i>gram</i>	<i>gram</i>	<i>mM</i>	<i>mM</i>	<i>mM</i>	<i>mM</i>
Control.....	24	410	14.1	19.1	24.4	40.1	34.0
S.D.....		26	0.9	1.9	4.3	3.3	2.9
350.....	2	398	13.6	19.0	21.9	43.8	32.9
370 + CO ₂	4	395	14.0	18.4	23.4	37.5	30.9

DISCUSSION. It should be borne in mind that the pressures studied are compatible with life in cats. The studies do not indicate that lower atmospheric pressures might not affect brain electrolyte but merely suggest such changes as occur in cerebral function at the levels studied are accompanied by only slight decreases in brain potassium. Furthermore, the changes are within the normal range in all cases.

It is known that the brain is more susceptible to low atmospheric pressures than other tissues. This conclusion is supported by changes in the electroencephalogram as well as mental tests. Improvement in the function as evidenced by these tests is obtained by adding carbon dioxide to the air at low oxygen tension (2). A loss of potassium in the brain is only strongly suggested by the present study and in any case is within the range of normal values and hence must be regarded as reversible. Hence, the studies do not give evidence that

change in brain electrolyte is an important feature accompanying the altered function of anoxia of a grade compatible with life.

The analyses of muscle confirm work in rats (3) showing no early change during short exposure to low atmospheric pressure. No previous results are available on hearts.

SUMMARY

Cats were exposed to low atmospheric pressure with and without the addition of carbon dioxide. Low atmospheric pressure leads to low serum bicarbonate in cats and this effect is more dependent on loss of sodium than increased concentration of chloride. Addition of carbon dioxide to atmospheres at low pressures prevents the decreased concentration of serum bicarbonate and sodium.

Analyses of the brain reveal a questionable reduction in the average figure for brain potassium. The decrease in brain potassium occurs at low atmospheric pressure with or without addition of carbon dioxide. This change is within the normal range and probably of little significance.

Heart and muscle show no changes in tissue water and electrolyte at the pressures studied.

REFERENCES

- (1) HARRISON, H. E. AND D. C. DARROW. *J. Clinic. Investigation* 17: 77, 1938.
- (2) BRAZIER, M. A. B. *Medicine* 22: 205, 1943.
- (3) DARROW, D. C. AND E. L. SARASON. *J. Clinic. Investigation* 23: 11, 1944.

DEPRESSION OF THE NORMAL ERYTHROCYTE NUMBER BY SOYBEAN LECITHIN OR CHOLINE¹

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In 1939 we reported that choline was effective in depressing the experimental polycythemia produced in dogs by either cobalt feeding or daily exposure to low atmospheric pressure, but that the administration of choline to normal dogs for 5 to 7 days was without effect on the normal red cell count (1). Later (2) we reported that soybean lecithin was capable of reducing experimental polycythemia in dogs. The mechanism of action of both substances was postulated to be a depression of red cell production by increased blood (and oxygen) supply to bone marrow, mediated by a vasodilator action of the drugs.

The purpose of the present investigation was to learn whether the prolonged daily administration of choline or lecithin for a period in excess of 5 to 7 days would depress the red cell count in normal dogs. This possibility did not seem unlikely, since bone marrow activity has a certain momentum or inertia which often requires considerable time for a change in rate of activity to be elicited. Certainly about 7 to 21 days may be required to produce experimental polycythemia by means of various agents (3-6).

METHODS. Red cell counts and hemoglobin percentages (Hellige) were determined regularly on normal dogs which were fed an adequate diet of Purina dog chow, and rolled oats. Occasional leukocyte counts and hematocrit determinations were also made.

After adequate control determinations had been made, the dogs were fed 5 grams of commercial soybean lecithin² daily. In the choline experiments, dogs were given 8 mgm. of choline hydrochloride per kgm. of body weight daily, by stomach tube (in dilute solution).

In all cases blood samples were drawn from the saphenous veins of the dogs while they were unexcited and in a fairly basal condition, at least 18 hours after previous feeding or drug administration.

RESULTS. Figure 1 shows the red cell counts of 4 normal dogs which were fed soybean lecithin daily. It will be seen that, after a latent period of 5 or more days, the erythrocyte numbers were gradually reduced. Maximal diminutions of 15 to 20 per cent were reached after 12 to 25 days of lecithin feeding. The long dashes in the line representing one dog (fig. 1) indicate a period during which 60 grams of lard were fed daily in addition to the lecithin. Cessation of lecithin feeding (short dashes) resulted in a return of the red cell counts of all dogs to normal within 11 to 20 days.

¹ Research paper no. 54S, Journal series, University of Arkansas.

² Soybean lecithin was generously furnished by the American Lecithin Company of Elmhurst, L. I., N. Y.

Figure 2 shows the erythrocyte counts of 4 dogs which received choline hydrochloride (8 mgm. per kgm.) in dilute solution by stomach tube. Two of the dogs received atropine sulfate (0.5 mgm. per kgm.) by stomach tube for 18 days (dashed lines) in addition to the choline. It will be seen that the two dogs which received choline alone showed significant reductions in erythrocyte counts after 15 days of choline feeding, while those receiving atropine in addition required about 10 days after atropine cessation, or a total of about 30 days to show comparable depressions. The atropine appears to have blocked the action of the choline. Hemoglobin and hematocrit percentages were reduced proportionately with the red cell counts by choline, but leukocyte counts did not change significantly (not shown). The return of the red blood cell counts to normal is not shown because it was desired to continue these dogs on a somewhat related experiment.

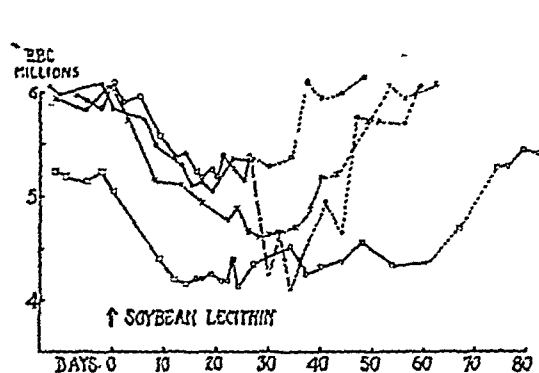


Fig. 1

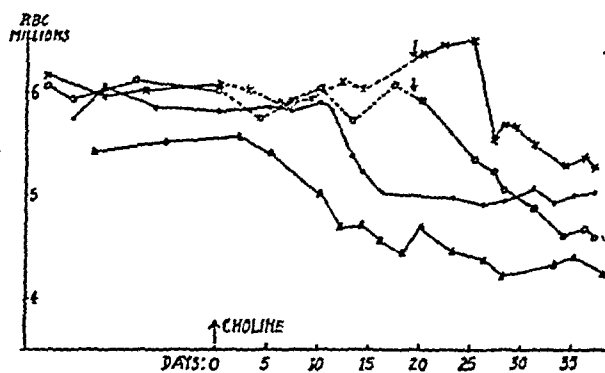


Fig. 2

Fig. 1. The effect of soybean lecithin on the red blood cell counts of four normal dogs. Dashes indicate cessation of lecithin administration. Long dashes in solid dot line indicate period during which 60 grams of lard was fed daily, in addition to the lecithin (5 grams daily).

Fig. 2. The effect of choline hydrochloride on the erythrocyte numbers of four normal dogs. Dashes indicate periods during which 0.5 mgm. per kgm. of atropine sulfate was fed daily, in addition to the choline chloride (8 mgm. per kgm., daily).

DISCUSSION. It would seem that choline and soybean lecithin probably lower the erythrocyte number by actually *depressing erythropoiesis*, if we may judge from the slow onset of choline action (fig. 2) and the slow recovery of the red blood cell count to normal after the cessation of soybean lecithin feeding (fig. 1). It is difficult to believe that other possible mechanisms such as hemolysis, blood dilution, or sequestration of erythrocytes in blood reservoirs could be concerned, —since they should not require 10 or more days to reduce the erythrocyte count to the extent shown in these experiments.

The time required for the mild anemia to develop in these experiments is comparable to that required in previous experiments to induce experimental polycythemia by various means (3-6). It seems highly possible that in both cases we are changing the rate of erythrocyte production—but in opposite directions.

The action of choline is a muscarinic action since it is antagonised by atropine in these experiments (fig. 2). Previously, we have shown that choline depressed

polycythemia probably by a vasodilator action, because its action was blocked by atropine, and because such vasodilators as nitrites and aminophylline were also effective in reducing certain experimental polycythemias (7, 8). Vasodilator drugs may depress erythropoiesis by improving the blood flow, and consequently increasing the oxygen supply, to bone marrow. Indeed, we have also approached this problem by increasing the *oxygen content* of arterial blood. We have depressed pituitrin-induced polycythemia in dogs by the administration of 100 per cent oxygen (by inhalation) for one hour daily (2).

As indicated in figure 1 by the long dashes, one dog apparently showed an extra reduction in erythrocyte count due to the feeding of fat (lard). It must be admitted, however, that three other dogs showed no decrease of erythrocytes when similarly treated. Our purpose in testing the effect of fat-feeding was to try to support the observations of Johnson et al. (9, 10) who, while unable to demonstrate a change in the erythrocyte number, have shown that the feeding of fat increases red cell fragility and causes increased erythrocyte destruction as judged by increased bile pigment output.

We assume that soybean lecithin probably affects the erythrocyte count by virtue of its choline content, which is placed at about 3 per cent (11).

CONCLUSIONS

The daily oral administration of 5 grams of soybean lecithin to 4 normal dogs caused significant reductions in their red blood cell counts, which persisted for at least 10 days after cessation of lecithin feeding.

Choline hydrochloride, in a daily oral dose of 8 mgm. per kgm. of body weight, caused significant depressions in the erythrocyte numbers of 4 dogs. More than ten days were required for maximum depression of red cells to occur, and atropine was shown to block the depression for a longer period than the 18 days during which it was administered.

These results are explained by assuming that choline depresses erythropoiesis by increasing the blood flow and oxygen supply to bone marrow—through its vasodilator action.

REFERENCES

- (1) DAVIS, J. E. This Journal **127**: 322, 1939.
- (2) DAVIS, J. E. J. Pharmacol. and Exper. Therap. **79**: 37, 1943.
- (3) DAVIS, J. E. Proc. Soc. Exper. Biol. and Med. **37**: 96, 1937.
- (4) DAVIS, J. E. This Journal **129**: 140, 1940.
- (5) DAVIS, J. E. This Journal **134**: 219, 1941.
- (6) DAVIS, J. E. This Journal **137**: 699, 1942.
- (7) DAVIS, J. E. J. Pharmacol. and Exper. Therap. **70**: 408, 1940.
- (8) DAVIS, J. E. J. Pharmacol. and Exper. Therap. **73**: 162, 1941.
- (9) LONGINI AND JOHNSON. This Journal **140**: 349, 1943.
- (10) LOEWY, FREEMAN, MARCHELLO AND JOHNSON. This Journal **138**: 230, 1943.
- (11) Amer. Lecithin Co. pamphlet, Soybean lecithin as a dietary source of choline.

PALMAR SKIN RESISTANCE (P.S.R.) DURING A STANDARD PERIOD OF CONTROLLED MUSCULAR ACTIVITY AS A MEASURE OF PHYSICAL FITNESS AND FATIGUE

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The present study was undertaken to determine whether in man quantitative changes related to fatigue and variations of fitness occur in the reactivity of the sympathetic nervous system as reflected in sweat gland activity and measured as palmar skin resistance (P.S.R.). Preliminary reports have been published (1).

The extensive literature on the subject of skin resistance has been reviewed by Landis and DeWick (2) and Landis (3). The realization of the recognized possibilities inherent in the nature of skin resistance has been handicapped by the large variabilities encountered in the measurements. A great increase in the reliability has been made possible in this study by improving the technique of the measurement itself and by making the measurements under the condition of a constant physiological stress of considerable magnitude.

That muscular activity reduces P.S.R. has been shown by Starch (4) and White (5), and that there is a general relation between the degree of muscular tension and the amount of decrease of P.S.R. has been shown by Wenger and Irwin (6) and Freeman and Simpson (7). Cannon (8) has shown that certain stress conditions, including muscular work, activate the sympatho-adrenal system. The extent of the drop of P.S.R. occurring at different times under the same work stress may then give an indication of changes in the functional response of the sympathetic system.

The stress period as a condition for determining significant changes of sympathetic reactivity was generally limited to one minute of work in order to minimize the secondary effects of metabolites and temperature changes. Additional work periods on the ergometer were employed to measure working capacity and to explore more fully the relationship of P.S.R. to work.

The bicycle ergometer. A bicycle was arranged to drive a separately excited electrical generator. The load condition was varied by changing the resistance in the generator circuit. The energy generated was measured by an ordinary dynamometer type watt-hour meter modified to measure units of one-hundredth of a watt-hour. A measure of the work done was thus obtained even with changes in speed of pedaling. The work done by the subject was the energy measured by the watt-hour meter plus the energy losses in the bicycle and generator.

In the experiments the subjects were instructed to maintain a speed which was approximately sixty-two revolutions of the pedals each minute, indicated by a voltmeter connected across the generator. When they could no longer maintain the speed, they were to pedal at the best rate possible.

Measurement of palmar skin resistance. Two shallow pans measuring about 25 by 35 cm. and containing zinc plates connected to a bridge circuit were fixed in place of the handle bars of the bicycle. For each experiment, gauze was laid on the plates and the pans were filled to a depth of about 6 mm. with an electrolyte, usually a 1 per cent solution of sodium chloride in tap water. The temperature, an important variable, was controlled by connecting a low voltage transformer, which supplied a safe source of current, to resistance wire arranged beneath the pans.

After mounting the bicycle the hands were placed palms down in the pans, where they remained during the experiment. Although pressure and movement of the hands did not appear to affect significantly the measurements of P.S.R.,

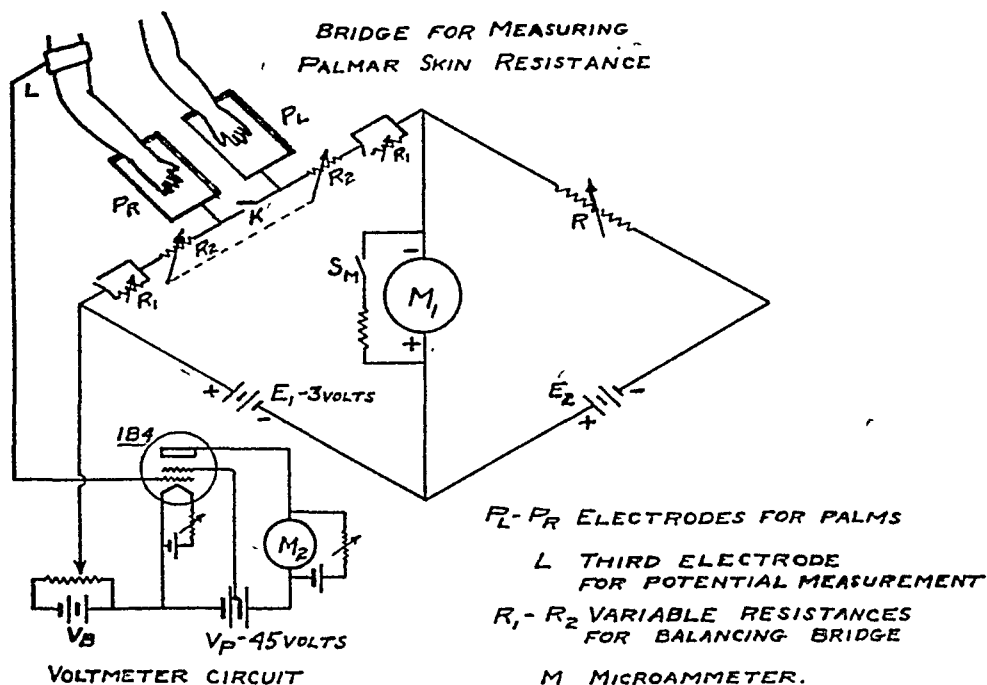


Fig. 1. Diagram of bridge circuit. The voltmeter circuit was used only to obtain the separate palmar resistances.

these conditions were controlled in most of the experiments by having sponge rubber pads on an adjustable bar press against the backs of the hands.

Besides the greater accuracy and sensitivity obtained by using the entire palmar surfaces as electrodes, the palmar resistance is always measured for identical areas.

The circuit used to measure P.S.R. is shown in figure 1 with an auxiliary voltmeter circuit used only to measure the relative resistances of the separate palmar areas. The simple circuit resembles that employed by Darrow (9), in that resistance can be added or subtracted in the subject arm of the bridge to maintain a relatively constant current through the subject. The current varied between 0.46 and 0.60 milliampere. The bridge was initially adjusted with 7000 ohms in the subject arm. Measurements were accurate to 20 ohms.

To measure the resistance in each palm, a vacuum tube voltmeter and means

for conveniently manipulating the external resistances R_1 and R_2 were added to the foregoing circuit. Thus the total resistance in the subject arm of the bridge could be made equal to a given amount, and point L between the two palms could be kept at the midpoint of the total resistance.

Subjects and conditions. The experimental data, except as otherwise indicated, were obtained from three groups of subjects. For each of these groups daily measurements for five days each week were made over a period of six weeks or more. The daily routine activities of each subject preceding the experiments were the same. All of the experiments were performed in the forenoon.

TABLE 1

Palmar skin resistance averages for each subject, before work and in the first, fourth and last work periods on bicycle ergometer

GROUP AND SUBJECT	NUMBER OF EXPERIMENTS	PSR AND STANDARD DEVIATIONS (SD) IN TENS OF OHMS											
		Before work				First work period				Fourth work period		Seventh work period	
		Initial		55* after initial		After 15* of work		Near end of period		Near end of period		Near end of period	
		PSR	SD	PSR	SD	PSR	SD	PSR	SD	PSR	SD	PSR	SD
1—W. C.....	20	496	146			405	116	387	113	377	101	325	77
*R. H.....	25	469	75			432	60	422	59	467	77	334	75
*M. N.....	20	450	33			376	27	378	31	383	20	349	14
G. T.....	20	370	45			325	29	327	29	366	25	344	26
F. S.....	20	358	45			276	26	285	24	318	21	279	18
H. N.....	20	360	39			326	41	314	44	297	43	253	25
2—R. S.....	20	277	26	331	36	253	30	219	29	189	13	176	11
A. D.....	12	269	42	392	78	309	73	260	52	200	13	177	11
D. X.....	20	328	30	432	61	310	45	286	34	270	23	244	21
*I. S.....	19	319	22	389	66	342	77	291	68	213	12	204	8
E. B.....	20	211	14	233	15	199	14	189	12	167	5	155	5
H. M.....	19	267	37	290	32	225	26	216	19	195	13	173	10
E. L.....	20	264	29	288	27	250	22	241	17	228	24	206	26
*A. L.....	20	347	21	375	33	301	25	288	27				
*A. N.....	20	381	40	441	56	390	73	317	54				
*M. H.....	19	285	24	303	32	249	27	247	22	249	18	237	16
3—H. L.....	20	300	45			262	37	257	28	194	18		
F. F.....	19	351	32			329	34	288	26	203	12		

* Women.

The six subjects of group 1 were obtained through a social service agency and had various employments in the afternoon and evening. The work experiment for subjects of group 1 consisted of seven work periods of one minute each, separated by rest periods of one-half minute. The load condition for each subject was constant from day to day. The palmar electrolyte was at room temperature, which varied between 66° and 74°F.

The ten subjects of group 2 were industrial employees, chemists and clerks, whose regular work was the same each day. The work experiment was the same as for group 1, except that the load for the first two periods was lighter so that a

constant speed could always be maintained for these periods. The palmar electrolyte was maintained at body temperature.

The two subjects of group 3 were students on vacation. Their conditions on the ergometer were the same as for group 2, but the experiment terminated after the fourth period of work.

Changes of P.S.R. during muscular work. Table 1 shows for each subject the averages and standard deviations of P.S.R. at certain points in the experiments. The average curves in figure 2 illustrate the variations of P.S.R. For each minute of work there are shown joined together three measurements, the first taken during the preceding non-working period, followed by P.S.R. at fifteen seconds of work and at forty-five or fifty-five seconds, the latter for group 2. All curves begin with the initial P.S.R., which was measured within five seconds after immersion of the palms. In the case of group 1 this measurement was the only one

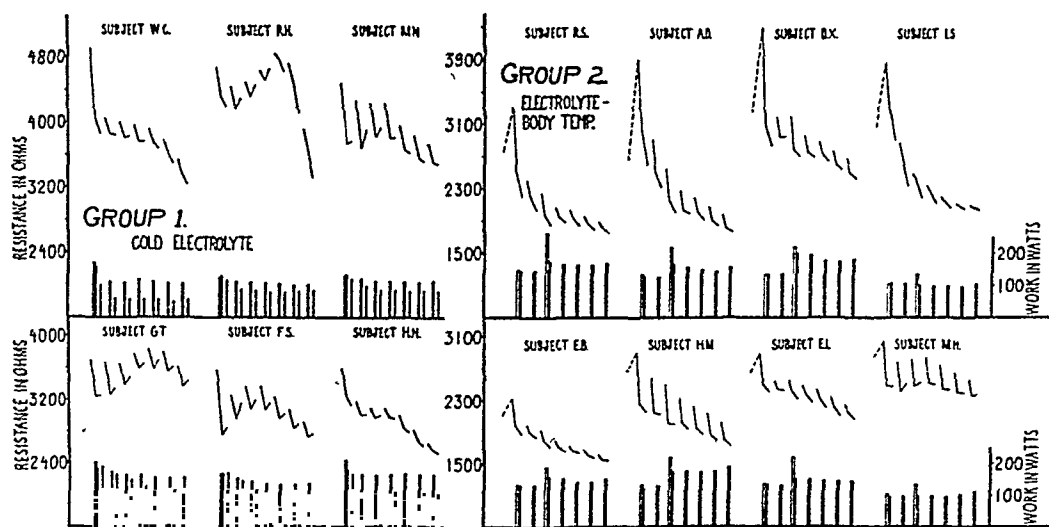


Fig. 2. Average P.S.R. for subjects of groups 1 and 2 during experiments of seven one-minute work periods, separated by rest pauses of one-half minute. Open bars indicate the work that could have been performed if the speed had been fully maintained.

made before work. For group 2, P.S.R. is also shown at fifty-five seconds after immersion, which was five seconds before work began.

With the onset of work a large initial decrease of P.S.R. occurred. The decrease was definite within five seconds and in some cases reached the maximal amount at the end of fifteen seconds, although it sometimes continued beyond the first minute of work. The level of P.S.R. attained during work depends on the load, as may be seen in group 2 (fig. 2) at the third period when the load was increased.

After the initial drop the level from period to period decreased at a slower rate or remained the same or definitely rose. Subsequent to the fourth minute of work the level in almost all cases decreased. The foregoing changes in level also occurred when the work was continuous for a period of ten minutes. In the experiments with alternate work and rest, P.S.R. with few exceptions rose during rest and dropped with work.

A rise in the trend of P.S.R. after the initial fall does not depend on a decrease in the working rate, since it occurred when the rate of work remained constant, as may be seen in figures 4C and 5. The final drop in the level beginning in the fourth to sixth work period is also not dependent on the rate of work, since it occurred with a constant, a rising, or a falling output. In other experiments work with relatively heavy loads for only two minutes was sometimes followed by decreases of resistance in the resting record six to eight minutes after the beginning of work. This point also corresponded to the time at which two subjects collapsed on the bicycle (fig. 4B). Subject R was able to remain for a short

TABLE 2

Correlation between day to day changes of P.S.R. and changes of work output on the bicycle ergometer

GROUP	SUBJECT	COEFFICIENTS OF CORRELATIONS			
		Between work done in the first five one-minute work periods* and P.S.R.			Between total work and P.S.R. measured at the end of the last work period
		Measured initially	Measured during the first work period		
			At 15 sec.	At 45 or 55 sec.	
1	W. C.	-0.42	-0.54	-0.55	-0.58
	R. H.	+0.47	+0.32	+0.23	-0.56
	M. N.	-0.17	+0.06	-0.26	-0.12
	G. T.	-0.42	-0.22	-0.28	-0.20
	F. S.	-0.16	-0.02	-0.32	-0.24
	H. N.	-0.36	-0.61	-0.59	-0.57
2	R. S.	-0.14	-0.39	-0.45	+0.11
	A. D.	-0.49	-0.32	-0.63	+0.09
	D. X.	-0.11	-0.06	-0.26	+0.20
	I. S.	-0.04	+0.32	+0.23	+0.26
	H. M.	-0.14	-0.66	-0.50	-0.35
	E. L.	-0.42	-0.41	-0.23	-0.21
3	M. H.	+0.09	-0.39	-0.50	+0.01
	H. L.	-0.32	-0.15	-0.56	+0.04
	F. F.	-0.53	-0.12	+0.04	+0.48
Subjects combined.....		-0.23	-0.25	-0.34	-0.14
t for coefficients.....		3.72	4.21	5.81	2.22

* Except for H. L. and F. F. who worked only four periods.

while on the ergometer after stopping work, and P.S.R. after a small rise dropped 700 ohms.

Relationship between P.S.R. and working capacity. Marked differences in the level of P.S.R. often occurred from one day to the next in the case of each subject. These differences occurred in the P.S.R. measurement made near the end of the first period of work, although the amount of work done in this period from day to day was fairly constant for each subject of group 1 and quite constant for subjects of groups 2 and 3, who had relatively lighter loads for the first two periods. The day-to-day changes of P.S.R. were found to correlate with day-to-day changes of total work output on the ergometer.

Table 2 shows the Pearson coefficients of correlation between the day-to-day changes of work and changes of P.S.R. for each subject, excepting A. L. and A. N., whose work records after the third period were unreliable because of muscular pains, and E. B., who gave evidence of an early arterial hypertension. His P.S.R. was unusually low and had relatively small variations. Employing the method of Fisher (10) the individual coefficients were combined and *t* values for measures of significance were obtained for the combined coefficients of correlation. The coefficients show that a negative correlation exists between day-to-day changes of ergometer output and day-to-day changes of three measurements, initial P.S.R., P.S.R. at 15 seconds of work, and near the end of the first work period; moreover, this negative relationship may be regarded as highly significant. The best measurement is the one obtained near the end of one minute of work. The initial P.S.R. is not a measurement made under conditions of relaxation or rest since it was made immediately after mounting the bicycle and was preceded by the activity of the day.

Ergometer performance as a measure of physical fitness or fatigue is known to be of limited value. However, over a period of time and with many subjects a general relationship does hold between physical fitness and output of work. Thus the correlation between work on the bicycle ergometer and measurements of P.S.R. in the first work period is assumed to indicate a relation between physical fitness and P.S.R. measured during a constant work stress.

The effect of marked loss of sleep on P.S.R. The unusual P.S.R. curve shown in figure 4A was obtained in the afternoon following a night during most of which the subject had worked at a fairly strenuous and trying task and after which he had had only three hours of sleep. The high level, particularly in the first three work periods where speed and load were practically constant, the retarded fall in the early part of the curve, and the relatively small rise in the first rest period seem to be characteristic after sleep loss. The load for this subject was over one and a half times that for most of the men of group 2, accounting for the relatively low values of P.S.R. throughout.

That a relationship exists between P.S.R. and sleep is indicated in that the subjects with greater variations in sleep show greater day-to-day variations in P.S.R. Between the average deviations of sleep of the subjects of groups 1, 2 and 3 and the standard deviations of P.S.R. measured near the end of the first work period, the correlation coefficient is 0.79 with a *t* of 4.9.

To illustrate the day-to-day relationship between sleep and P.S.R., figure 3A presents for four subjects of group 2 for a period of nine weeks daily records of P.S.R. after 55 seconds of work and amounts of sleep for the preceding night.

Subject A. D. had no sleep and was under strain on the nights of November 7th and 14th. After each sleep loss his P.S.R. became unusually high. In the first instance, P.S.R. rose to 3740 ohms and in the second to 3840 ohms. Each rise was followed by a decrease with sleep. His normal P.S.R. as indicated by the average during the period prior to the sleep losses, October 6th to 17th, was 2073 ohms.

Subject I. S. showed the same effects of sleep loss. In one instance after loss of sleep P.S.R. rose to 4860 ohms and in another to 4060 ohms. With better

sleep, P.S.R. returned toward her comparatively rested resistance level of about 2200 ohms. Similar effects of sleep loss were observed in other subjects.

Contrasted with A. D. and I. S., subjects E. L. and A. L. had smaller fluctuations in sleep and correspondingly smaller fluctuations in P.S.R. Moreover, during the period when their sleep was similar their P.S.R. records showed a

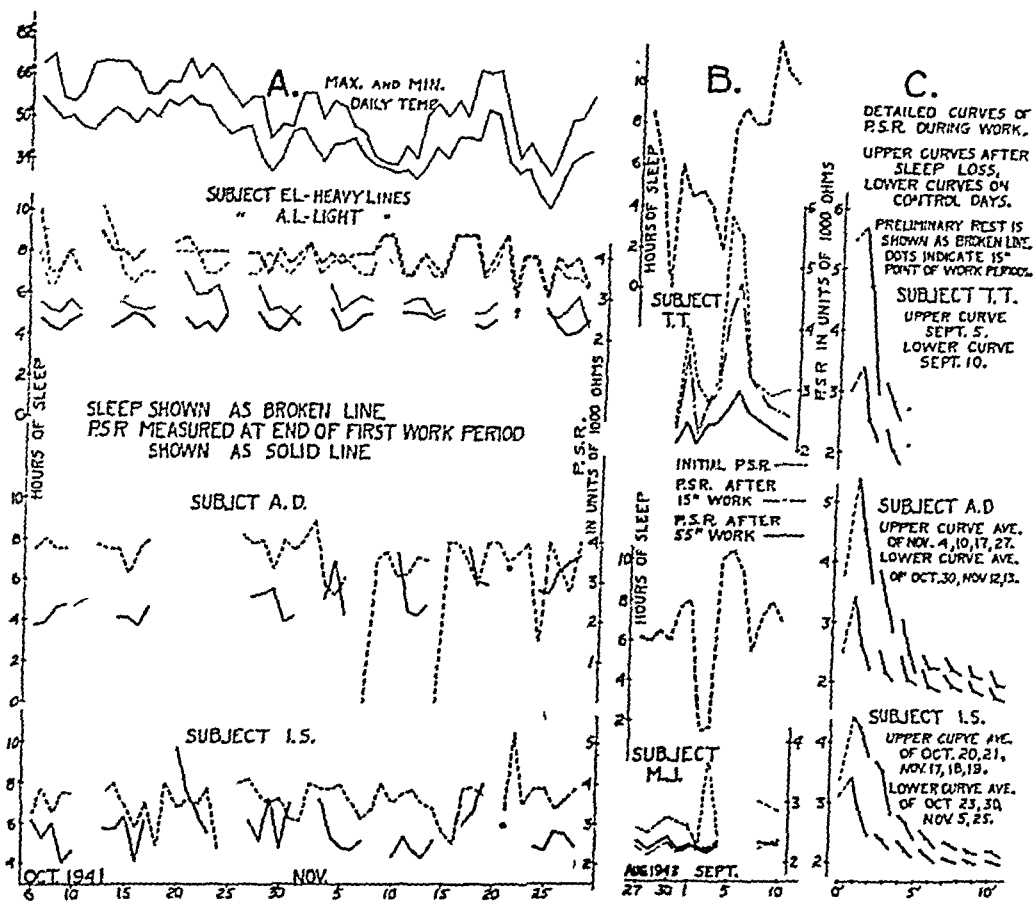


Fig. 3. Effect of marked sleep loss on P.S.R.

A. Two subjects, A. D. and I. S., had marked sleep losses, and two subjects, E. L. and A. L., with less sleep losses serve as controls. Large increases of P.S.R. occurred after sleep loss followed by decreases with sleep.

B. Experiment with two subjects. Marked increases of P.S.R. occurred after large sleep losses.

C. Detailed records of P.S.R. obtained during work experiments on three subjects for days with sleep losses and for control days. See also figure 4A.

marked resemblance. Also during the period when A. L. obtained less sleep than E. L. her record was at a relatively higher level.

The averages of the lowest 20 per cent of the P.S.R. measurements for individuals of group 2 made near the end of the first minute of work ranged from 1735 ohms to 2290 ohms for the men and from 2115 ohms to 2545 ohms for the women. In no case did the low records fall on or immediately follow days when the sleep was less than the subject's average amount. The averages of the highest twenty per cent of the records exceeded 3000 ohms for five subjects, ranging from 3205

ohms to 4208 ohms. For four of these subjects, A. D., D. X., I. S. and A. N., the only ones who had very large sleep losses and who frequently reported being very tired, the records were obtained during periods of these losses. In the case of the fifth subject, A. L., the records were obtained after less than her average sleep.

The evidence indicates that individual differences in P.S.R. measured under a standard work stress are much less between comparatively rested subjects than differences occurring in the same individual between rested and fatigued states. The subjects of groups 1 and 3 cannot be included in this consideration

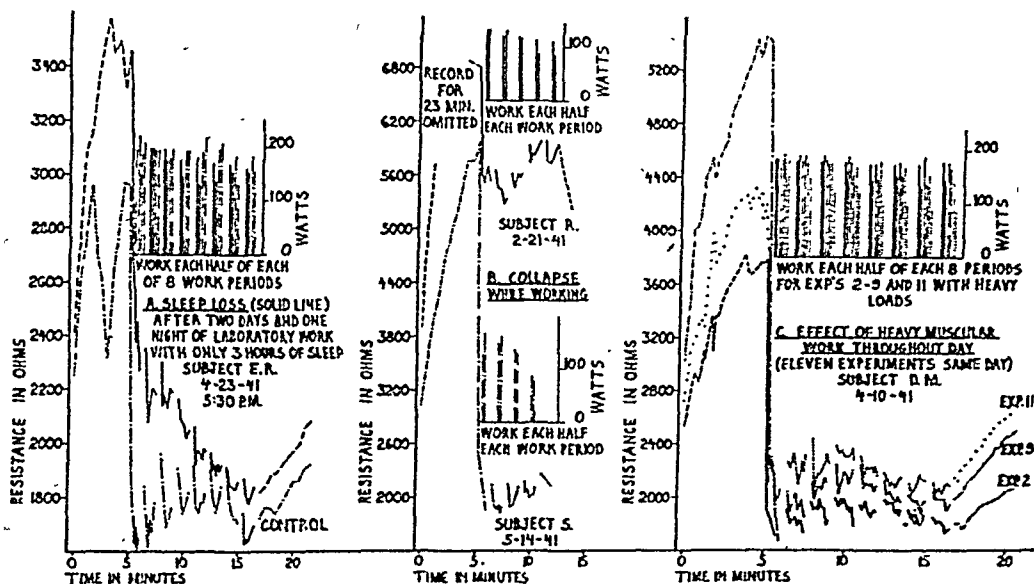


Fig. 4. A. Record of P.S.R. and work under the condition of heavy load after a marked sleep loss. Although little difference occurred in the work performed in the first two work periods, P.S.R. was much higher after loss of sleep.

B. P.S.R. and work records of two subjects who collapsed during experiments on the bicycle ergometer. With collapse, a sharp drop of P.S.R. occurred. For subject R the electrolyte was zinc sulphate, accounting for the high P.S.R. level.

C. Work experiments, each consisting of eight one-minute work periods, were repeated throughout the day with rest intervals of 18 minutes. In the 11th experiment P.S.R. was highest, even in the first work period when work was constant.

of individual differences because a cold electrolyte was used with group 1 and no records of week-end sleep and activities were kept for either group.

A direct experiment on sleep loss in relation to P.S.R. was performed on two subjects who voluntarily deprived themselves of sleep. Their daily employment was much less severe than for subjects of group 2, and no stress occurred with the loss of sleep. Figure 3B shows for each subject the daily amounts of sleep and three daily P.S.R. measurements, initial P.S.R. one minute before work and P.S.R. after fifteen seconds and after fifty-five seconds of work.

Subject T. T., beginning with an all-night automobile drive and no sleep, obtained only twenty-one hours of sleep for a six-day period. Then for the following six days he obtained fifty-five hours of sleep. Each P.S.R. measurement became higher during the period of sleep loss and continued to rise after

two nights with fairly good sleep. Not until after three nights of sleep of about eight hours each did the P.S.R. records begin to decrease and recovery continued through four additional days, when the record ended.

The P.S.R. record for M. J., whose sleep loss was much less than that of T. T., showed considerably less change. The record for the period following sleep loss was incomplete.

Average curves of P.S.R. for subjects A. D., I. S. and T. T. under conditions of normal sleep and sleep loss are shown in figure 3C. A definite resemblance exists between these curves and that of figure 4A.

The effect of sleep losses over the two-day week-end holidays on P.S.R. compared with the effect of usual or better than usual amounts of sleep is summarized

TABLE 3

Effect of week-end holidays on fatigue level of employed workers as indicated by P.S.R.

Average P.S.R. at 55 seconds of work is shown for Monday and the change from the preceding Friday. Average sleep per night is shown for three nights of the week-end. A decrease of P.S.R. indicates less fatigue and a rise, greater fatigue.

SUBJECTS	WITH BETTER THAN USUAL REST			WITH UNUSUALLY LARGE SLEEP LOSSES			WHEN OVERNIGHT TRIPS WERE MADE			ALL OTHER WEEK-ENDS		
	Monday	Change from Friday	Ave. sleep	Monday	Change from Friday	Ave. sleep	Monday	Change from Friday	Ave. sleep	Monday	Change from Friday	Ave. sleep
R. S.	2080	-440	7.00				2220	+83	7.25			
A. D.	2630	-310	8.00	3790	+1480	5.57				2497	+37	7.10
I. S.	2470	-490	8.60	3910	+810	6.40	2840	+520	7.25	2910	-130	7.50
E. B.	1860	-160	9.00	2080	+200	6.66	1950*	+180	8.20	1800	-163	8.10
H. M.	2145	-225	8.75				2360*	+300	8.00	2143	+61	8.25
E. L.	2265	-245	8.85	2650	+400	7.66	2420	+200	7.90	2633	+16	7.90
M. H.	2310	-310	9.00							2550	-72	7.75
A. L.	2585	-70	9.00	3230	+530	7.33	3490	+860	7.66	2893	+230	7.66
A. N.	3160	-70	8.00	3860	+1390	6.66				3120	+140	7.38
Averages		-257	8.49		+802	6.71		+357	7.78		+15	7.71
t (Fisher)		3.26			4.26			2.86			0.30	
No. of items...		14			8			9			30	

* Period is from day before to day following a one-day trip between Monday and Friday.

in table 3. The table includes the data for all week-ends for subjects of group 2 for whom records were available. With better than usual sleep or rest over the week-end holiday there was for every subject a fall in the level of P.S.R., but an unusually large sleep loss was always followed by a marked rise of P.S.R. The week-end change of P.S.R. for each condition is statistically significant.

Effect of muscular fatigue on P.S.R. Experiments consisting of two-minute work periods with rest intervals of five, ten, fifteen and thirty minutes respectively between successive work periods were performed on the bicycle ergometer. The work was done at a constant speed and with the heaviest load which each subject could maintain.

Figure 5 illustrates the results of four of the five experiments on two subjects.

There is a remarkable similarity between the P.S.R. records obtained on the two subjects and between experiments separated by a month.

The most pronounced effect of previous work on P.S.R. measured during subsequent work occurred after a rest period of ten minutes, but the effect did not fully disappear after thirty minutes of rest. The difference between the average of the lowest P.S.R. measurements in the first work period and in the work period after ten minutes of rest is 450 ohms, which is statistically very significant with a t of 7.3 for five experiments. A computation of the average of the lowest P.S.R. records obtained in each work period for the five experiments gives the following values with their standard deviations; 1st period 1746, S.D. 105; 2nd period 1810, S.D. 71; 3rd period 2200, S.D. 39; 4th period 2170, S.D. 158; and

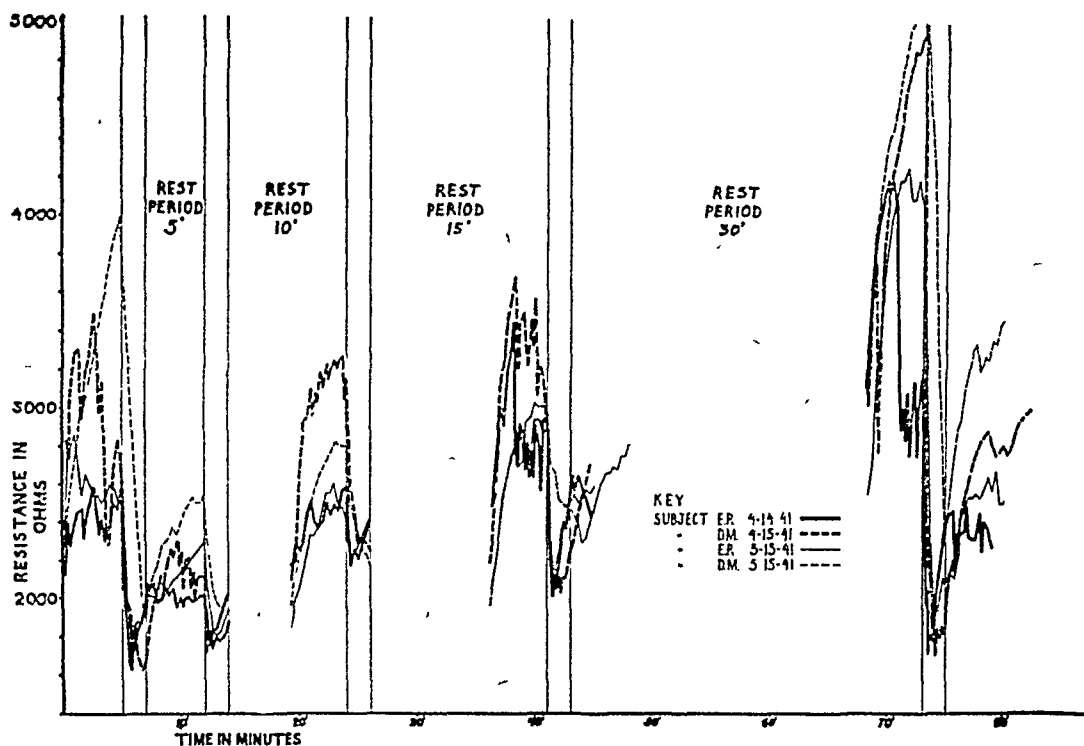


Fig. 5. After-effects of work on P.S.R. Work periods of two minutes with a constant heavy load and speed were separated by rest periods of varying amounts.

5th period 2060, S.D. 333. The low standard deviations are an indication of the reliability obtainable with the method. The variability between experiments is least in the third work period after a ten-minute rest pause. It is of interest in this connection to note that Foltz, Ivy and Barborka (11) found less variability in work performed on the bicycle ergometer in a second work period performed after ten minutes of rest than in the first work period.

In another experimental series a subject was required to work throughout the day until a definite decrease due to fatigue occurred in the work output. Eleven experiments were performed. Each experiment consisted of eight one-minute periods of work with half-minute rest pauses and with eighteen minutes of rest between experiments except for lunch.

Figure 4C shows P.S.R. during work periods and rest intervals for the second, ninth and eleventh experiments of the day and the relative rates of work for each half of each work period. The general level of P.S.R. was lowest when the subject was comparatively fresh in the second experiment. In the eleventh experiment, in which the total work output was less by fifteen per cent and the subject reported being very tired, P.S.R. was much higher, even in the first work period in which the rate of work was unchanged.

Influence of electrolyte on P.S.R. The necessity for considering the kind of electrolyte in the technique of measuring P.S.R. in order to obtain reliable measurements is brought out in a comparative study of the effect of different electrolytes on P.S.R.

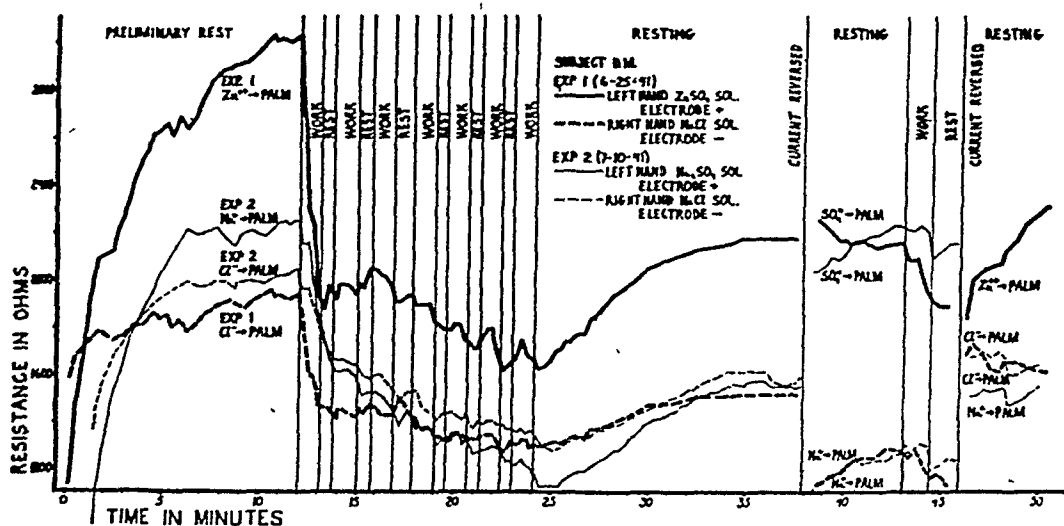


Fig. 6. Comparative effects of different electrolytes on P.S.R. during rest and work. The resistance was higher when the current was carried to the palm by Zn^{++} ion in comparison with the Na^{+} ion and by the SO_4^{--} ion in comparison with the Cl^{-} ion.

In two different work experiments, sodium chloride was used as the electrolyte for the right hand. For the left hand zinc sulphate was used in the first experiment and sodium sulphate in the second. The direction of the current determined which of the external ions passed through the skin. The results of the experiments are shown in figure 6. Initially, before the external ions could migrate to the barrier membranes, the resistances were not greatly different. With time, however, the resistance to zinc ions increased much more rapidly than to chlorine or sodium ions. With reversal of the current the resistance to sulphate ions became similar to the resistance to zinc ions and much higher than to sodium ions.

The explanation for the greater fall in resistance with work when the zinc ions carry the current through the skin may be that the sweating to some extent carries the zinc electrolyte away from the sweat glands, and the electrolyte becomes that of the sweat itself.

SUMMARY

With the entire palmar areas in an electrolyte of sodium chloride at body temperature, reliable measurements of palmar skin resistance were obtained, especially under conditions of a standard work stress on the bicycle ergometer.

The palmar skin resistance decreased within five seconds after the beginning of muscular activity on the bicycle ergometer, and there was a large decrease in the first minute of work. In experiments with seven or eight work periods, there was a stage during which the general trend of palmar skin resistance either decreased at a slower rate, remained level, or rose, followed by a stage in which there was a final downward trend.

A significant negative correlation was found between day-to-day changes in the level of palmar skin resistance measured near the end of the first minute of work and working capacity measured by the total work performed on the ergometer.

Large sleep losses resulted in marked increases of palmar skin resistance measured in the first minute of work. Recovery with normal sleep was not immediate. Heavy muscular work without an adequate period for recovery likewise raised the level of palmar skin resistance.

The level of palmar skin resistance measured near the end of one minute of work under constant conditions of speed and load, constituting a standard work stress, is related to fatigue and physical fitness. Conditions producing fatigue are associated with higher levels of palmar skin resistance.

We wish to express our thanks to Profs. A. B. Luckhardt, N. Kleitman and Clyde Brooks for their helpful criticism and suggestions.

REFERENCES

- (1) RYAN, A. H. AND E. L. RANSEEN. *This Journal* **133**: P434, 1941; *Federation Proc.* **3**: 40, 1944.
- (2) LANDIS, C. AND H. N. DEWICK. *Psychol. Bull.* **26**: 64, 1929.
- (3) LANDIS, C. *Psychol. Bull.* **29**: 693, 1932.
- (4) STARCH, D. *Psychol. Rev.* **17**: 19, 1910.
- (5) WHITE, M. M. *J. Exper. Psychol.* **13**: 267, 1930.
- (6) WENGER, M. A. AND O. C. IRWIN. *Univ. Iowa Studies* **12**: 143, 1936.
- (7) FREEMAN, G. L. AND R. M. SIMPSON. *J. Gen. Psychol.* **18**: 319, 1938.
- (8) CANNON, W. B. *Physiol. Rev.* **9**: 399, 1929.
- (9) DARROW, C. W. *J. Gen. Psychol.* **6**: 471, 1932.
- (10) FISHER, R. A. *Statistical methods for research workers*. Edinburgh, 1938, Oliver and Boyd.
- (11) FOLTZ, E., A. C. IVY AND C. J. BARBORKA. *This Journal* **136**: 79, 1942.

RESPONSES OF BLOOD CAPILLARIES TO ACUTE HEMORRHAGE IN THE RAT¹

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Our previous studies (1, 2) have shown that the capillary bed possesses a well-defined architectural pattern of differently structured components and that the circulation through the bed is conditioned by the interrelated functional activities of these components. The significance of the capillary bed as an integrated functional unit in the peripheral circulatory failure following graded hemorrhage has been discussed in our recent publication (3).

This paper deals with an observational study of the changes which occur in the capillary bed as a result of varying degrees of acute hemorrhage. The observations present data by means of which it is possible to distinguish between the effects of blood-loss *per se* and those resulting from the existence of a prolonged hypotensive state, as shown in our publication cited above. A need for such data has been repeatedly stressed by Wiggers (4). The rat was selected and observations were made simultaneously on the mesenteric circulation in the mesoappendix and on the cutaneous circulation in the interdigital web of the hind foot.

MATERIALS AND METHODS. Male and female rats (Wistar strain) of about 125 to 150 grams in weight were used. The rats were deprived of food for about 10 hours but were allowed to drink water freely. The bleedings were carried out while the rats were anesthetized by the subcutaneous administration of sodium pentobarbital (2.5 to 3.5 mgm./100 grams body weight) in order to permit exposure of the mesoappendix.

Two microscopes were used for the observations which were made with transmitted light. One was for the mesoappendix, exposed according to a method previously described (4), and the other for the interdigital web of the hind foot. The cutaneous vessels were observed by using a depilatory to remove the scurf of the epidermis and applying an indifferent oil (paraffin oil) to increase the translucency of the skin. The interdigital web was used both for studying the cutaneous circulation and for taking blood pressure readings by the thigh pneumatic pressure cuff method (5) for occluding the femoral artery.

The studies were made in three categories of bleeding volumes, in ranges of 1.0 to 2.0, 2.0 to 2.5 and 3.0 to 3.5 per cent, the bleedings being recorded in terms of percentage of the body weight. In the majority of the experiments the bleeding was done through the severed stump of the tail immersed in a graduate cylinder.

¹ The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and New York University. It was also supported in part by the Eli Lilly Research Laboratories and the Josiah Macy, Jr. Foundation. It constitutes the fourth of a series of investigations on experimentally induced shock. The first is no. 2; the second, no. 5; the third, no. 7, in the bibliography of this paper.

der containing warm, isotonic sodium oxalate. By this method an amount equal to 2 per cent of the body weight could be removed in about $1\frac{1}{2}$ to 2 minutes and 3 per cent in about 4 to 5 minutes. The induced vasoconstriction tended to prevent the removal of larger amounts. This could be generally overcome by massaging the tail, which, however, frequently prolonged the bleeding time to about 8 to 10 minutes.

A few rats in each bleeding volume category were bled very rapidly by way of the femoral artery. The artery, exposed in the groin, was incised and the blood allowed to flow as rapidly as possible into a measured amount of sodium oxalate in an artificially exposed pocket in the groin. By this means it was possible to procure a blood-loss of 3 per cent of the body weight within 30 to 40 seconds. No differences were detected in the vascular responses to the two types of blood-loss.

The vascular components of the capillary bed in the interdigital web are distinguished readily but more fragmentarily than in the mesoappendix. Moreover, the cutaneous circulation becomes ischemic very early after hemorrhage and thereby greatly increases the difficulty of following the responsiveness of the vessels. In contrast to this, the circulation in the mesoappendix is maintained considerably longer and the vessels remain clearly visible all through the syndrome. For this reason most of the emphasis on the details of the post-hemorrhagic changes in the capillary bed which are given in this paper has been on observations of the mesoappendix.

The exposed mesoappendix was constantly irrigated with a drip of warm gelatin-Ringer's solution (2). Under these conditions it was possible to observe selected vessels of the bed continuously over a period of hours with no evidence of trauma incidental to exposure of the tissue.

The observations were begun before the bleeding and were continued through the hemorrhagic syndrome. Several criteria, described in our previous publication, were used to indicate the state of the capillary bed. These included: *a*, rate and distribution of capillary flow; *b*, arteriolar and venular caliber; *c*, vasomotion of the metarterioles and precapillaries, and *d*, reactivity of the metarterioles to the topical application of a few drops of epinephrine in gelatin-Ringer's solution on the surface of the mesoappendix. Each criterion was followed on a given vessel or set of vessels throughout the experiment. The epinephrine reaction was quantitated by noting the minimal concentration which produced a narrowing of the metarterioles sufficient to reduce but not stop the flow. The gelatin-Ringer drip was stopped during the period of the epinephrine test. Between successive tests an interval of at least 1 to 2 minutes was allowed during which the mesoappendix was thoroughly irrigated with the gelatin-Ringer drip.

EXPERIMENTAL RESULTS. A series of 140 rats were subjected to different degrees of blood-loss while under nembutal anesthesia. The accompanying table summarizes the relationship between the percentage of blood-loss and the percentage and extent of survival for this series. Under the conditions of these experiments, it was found that a blood-loss of between 3.0 and 3.5 per cent of the body weight brought about death in over 80 per cent of the rats within 40 to 60 minutes, and that rats bled more than 3.5 per cent invariably died within 25

to 35 minutes. Furthermore, it was found that rats bled to 2.5 per cent or more of their body weight always died provided the blood pressure had remained below 60 mm. Hg for about 30 to 60 minutes from the time of the bleeding.

The skin circulation was consistently curtailed by a blood-loss of 1 per cent and greater, irrespective of whether the rats died or survived. On the other hand, the mesenteric circulation showed little change until the blood-loss approached 2 per cent and became markedly curtailed only in those rats that died. In those rats which survived, the circulation was characterized by a persistence of the capillary flow which, although slowed, never lost its normal directional flow. In those rats which eventually succumbed, the slowing of the capillary flow was accompanied by a characteristic sluggish state of the venular flow and a progressive intensification of a backflow and trapping of blood in the capillaries and the venules.

Plate. Reaction of vessels in rat mesoappendix to acute hemorrhage (figures from microcinematographs).

Effect of moderate blood loss (2 per cent body weight)

Fig. 1. Before bleeding. Upward arrow, on non-muscular venule ($40\ \mu$); downward arrow, on arteriole ($25\ \mu$). Metarteriole branching off at right.

Fig. 2. After bleeding. Arteriole partially contracted, with thickened wall. Metarteriole not affected. Venule congested.

Asphyxial response five minutes before death after blood-loss (3 per cent body weight)

Fig. 3. Arteriole and metarteriolar branch markedly constricted and visible only as narrow cords with a few red cells in almost obliterated lumen. Non-muscular venule (to left of figure) is not contracted but is poorly visible.

Effect of marked blood loss (3.3 per cent body weight)

Fig. 4. Before bleeding. Traversing field from left to right (with arrow) is a metarteriole ($15\ \mu$). Branching down from it at the left is a precapillary offshoot leading into a true capillary partly covered with fat globules. To right of figure is a muscular venule with two capillaries flowing into it.

Fig. 5. Immediately after bleeding. Metarteriole, diameter unchanged, with slowed flow evidenced by visibility of individual blood cells. Muscular venule distended and congested.

Fig. 6. One hour after bleeding. Metarteriole with caliber still unchanged but flow irregular and pulsatile. Muscular venule much distended because of backflow. Venous end of capillaries distended by backflow of blood from venule.

Responses to topical application of epinephrine (magnification same as in fig. 1)

Fig. 7. Normal state. Arteriole ($25\ \mu$) coursing diagonally downward. Muscular venule at upper right of figure with non-muscular venule flowing into it.

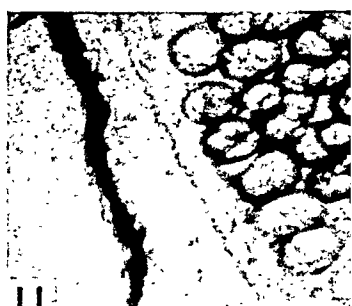
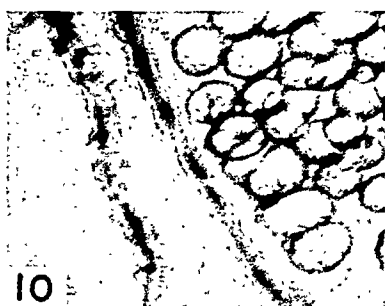
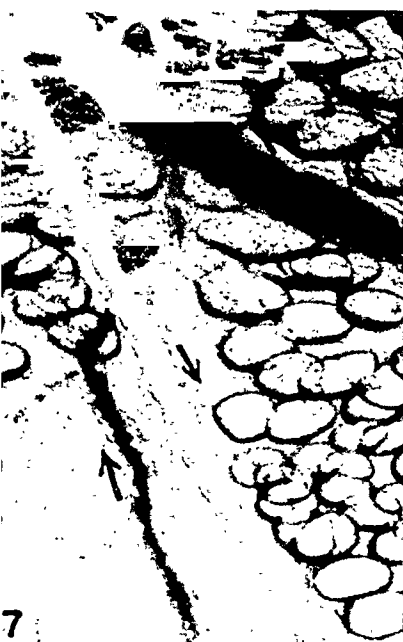
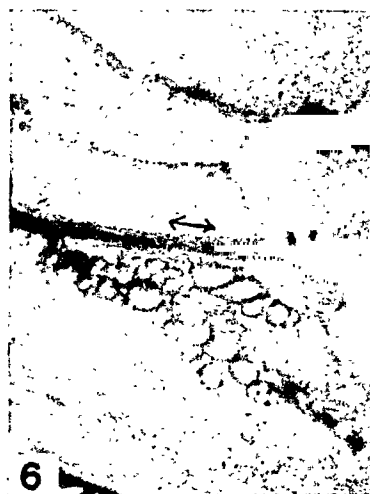
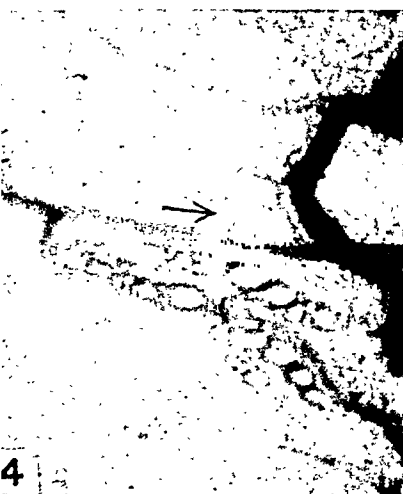
Effect of topical application of epinephrine (1:2 million) on vessels before (figs. 8 and 10) and after (figs. 9 and 11) a blood-loss of 2.5 per cent. (Note that non-muscular venule is unaffected in caliber in either condition.)

Fig. 8. Before bleeding. Muscular venule has reacted to the epinephrine by uneven, partial constriction.

Fig. 9. Immediately after bleeding. The same venule as in figure 8 has not reacted to the epinephrine. (Slightly narrowed caliber, compared with Fig. 7, was already present before application of the epinephrine.)

Fig. 10. Before bleeding. Arteriole has reacted by a partial constriction.

Fig. 11. After bleeding. Arteriole has reacted by complete constriction, indicating hyper-reactivity after bleeding.



I. *Responses of the Capillary Circulation.* The progressive changes in the peripheral circulation are described under separate headings, each representing a specific degree of blood-loss. The changes brought about by the bleedings in the different categories were cumulative. Because of this and in order to avoid repetition, the description given for the successively larger bleedings includes only the changes observed additional to those already described for the smaller bleedings. The chief difference observed was that, the greater the percentage of blood-loss, the shorter was the period occupied by the initial changes.

The following descriptions refer to the changes characteristic of the majority of animals in each category. The sections dealing with a blood-loss of 1 to 2 per cent and of 2.0 to 2.5 per cent treat only of those rats which survived, while the section on blood-loss of 3.0 to 3.5 per cent treats only of those which died of circulatory failure. Text figure 1 is a diagram of a functional unit of the capillary bed together with the outstanding changes of four criteria following blood-loss. Throughout this paper the observations were confined for the most part to the

TABLE 1

The table shows the different degrees of blood-loss to which the 140 rats were subjected. In each category are indicated the number of survivals and the length of time following blood-loss before fatal circulatory failure occurred

BLOOD-LOSS BY PERCENTAGE OF BODY WEIGHT	NO. OF RATS	NO. OF DEATHS	BLOOD PRESSURE 20 MINUTES AFTER BLEEDING	SURVIVAL TIME OF FATAL CASES
<i>per cent</i>			<i>mm.</i>	<i>min.</i>
1	15	0	85-90	•
2	30	3	70-80	120 \pm 20
2.5	32	14	50-60	75 \pm 15
3-3.5	55	45	40-45	50 \pm 10
4	8	8	30-35	20 \pm 5

vessels indicated in the figure. The changes for arteries up to 200 to 250 μ were found to be the same as those listed in the subdivision for the arteriole. The caliber changes for the muscular venules and veins (100-300 μ) are less striking than those for the arteries but are in the same direction. References in the paper to the capillary circulation *per se* include the other four categories of vessels listed in the figure.

a. *Blood-loss of 1 to 2 per cent.* The rat readily withstood a blood-loss of 1 per cent or less, no fatalities occurring in this category and no significant changes being observed in the blood vessels, either in the skin or in the mesentery. Out of a total of 45 rats subjected to 1 to 2 per cent blood-loss, 42 showed spontaneous recovery and, although a significant depression of blood pressure and peripheral blood flow occurred, both blood pressure and flow returned to normal within 60 to 90 minutes. The blood pressure in these rats showed an initial decline within 10 to 15 minutes after bleeding, levelling off at about 65 to 80 mm. Hg, but gradually returned to normal during the ensuing hour. The three rats which died showed a precipitous decline in blood pressure and stopped breathing about 12 to 20 minutes after being bled.

Cutaneous. The earliest peripheral vascular manifestation was the development of an ischemia in the skin as a result of a partial vasoconstriction of the larger blood vessels. This was accompanied by a marked narrowing of the arterioles (30μ) in the subcutaneous layer and their precapillary branches (15μ) leading into the subpapillary plexus of capillaries. The only flow discernible in the skin was in the deeply placed vessels which interconnect the arterioles and venules.

Mesenteric. This circulation showed no change except for a slight slowing in the rate of capillary flow, discernible chiefly on the venous side of the bed. No

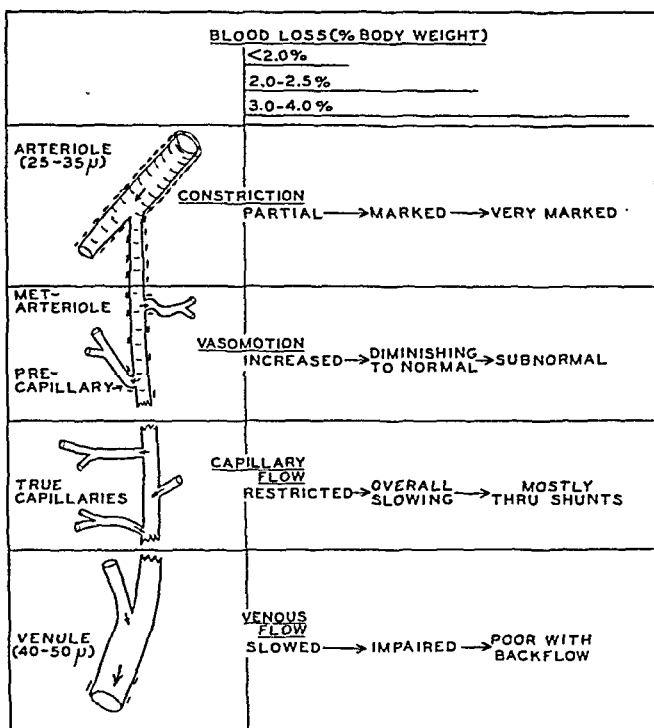


Fig. 1

Fig. 1. Diagram of a central channel leading from arteriole through capillary bed to venule. Listed are responses of the several components of the capillary bed to varying degrees of hemorrhage. On top of chart the horizontal lines, placed under each given degree of blood-loss, indicate the extent to which the changes placed below are carried. The rest of the chart is subdivided into several vascular components. Changes in a specific function of each are indicated following a given blood-loss.

Fig. 2. Diagram indicating principal changes in peripheral circulation following acute hemorrhage.

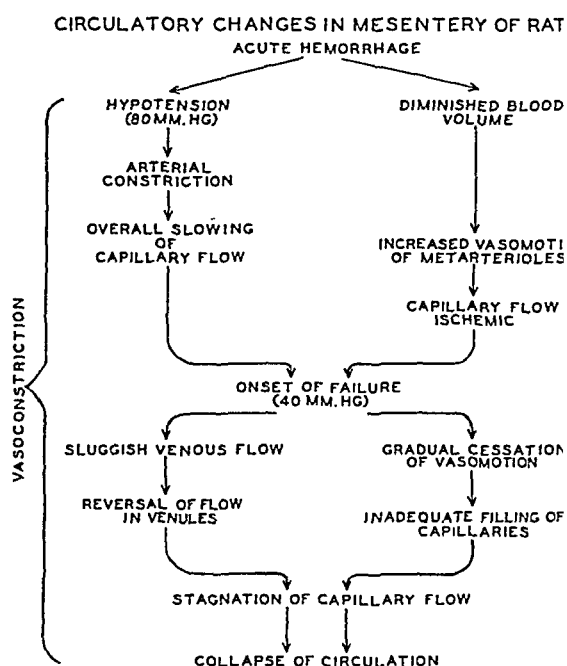


Fig. 2

change occurred in the caliber of the larger arteries ($100-150\mu$) and veins ($150-250\mu$) in the mesoappendix.

b. *Blood-loss of 2.0 to 2.5 per cent.* A blood-loss of 2.0 to 2.5 per cent of body weight represents the intermediate range in which statistically significant numbers of deaths occur. Out of a total of 32 rats bled 2.5 per cent of their body weight, 18 survived. This also represents the range in which well-defined changes occurred in the mesenteric capillary circulation.

Cutaneous. Within 5 to 10 minutes after the onset of bleeding all the muscular vessels underwent complete vasoconstriction and the subpapillary plexus be-

came completely ischemic. This state was maintained throughout the rest of the syndrome.

Mesenteric. These vessels underwent progressive changes in flow and reactivity throughout the syndrome. When the blood-loss reached 2.5 per cent, the arterioles narrowed markedly, e.g., one of 35μ constricting to 20μ . A few minutes later, the larger arteries constricted to about half their original caliber. These changes, combined with the ensuing hypotension (50 to 60 mm. Hg), were accompanied by a slowing of flow in the capillaries. The metarterioles exhibited increased vasomotion but never underwent the prolonged and generalized constriction characteristic of the larger vessels. The contrast between the arterioles and their metarteriolar branches at this stage is shown in plate figures 1 and 2. The absence of constriction of the metarteriole is also shown in plate figure 5. It should be noted, as shown in the various photographs of the mesenteric vessels, that no active contraction ever occurred either in the true capillaries or in the non-muscular venules.

The blood pressure rarely fell below 50 mm. Hg and no backflow or stagnation developed in the mesenteric capillary bed circulation. A marked slowing of flow was especially evident on the venous side of the bed and resulted in an overfilling of the non-muscular venules. However, in the larger veins an adequate flow was still maintained because of a flow through direct arterio-venous anastomoses between arterioles and venules. During this time the flow through the capillary vessels became increasingly restricted to the central channels of the capillary bed (partial ischemia), until only about one-third of the true capillaries contained an active circulation. This restriction of the capillary circulation was accompanied by an increased vasomotion of the metarterioles and precapillaries. The frequency of the alternating dilator and constrictor phases increased within 5 to 10 minutes after the bleeding and was rapidly followed by a condition in which the constrictor phase progressively became more prominent. Constriction of the precapillary sphincters confined the flow to the central channels from metarteriole to venule. During the brief dilator phases the opening of the precapillary sphincters resulted in a periodically recurrent flushing of blood through the capillary bed so that the ischemia was intermittent. The true capillaries always remained open even during the relatively long constrictor phase of the metarterioles and precapillaries and continued to drain into the distal end of the flowing a-v channel and thence into the venous circulation.

c. *Blood-loss of 3.0 to 3.5 per cent.* In this group about 80 per cent of the rats died, the usual survival time being 45 to 50 minutes. With this excessive blood-loss (3 per cent and greater), the blood pressure fell precipitously to about 60 mm. and in the fatal cases steadily dropped during a subsequent period of 20 to 30 minutes to a level of about 40 mm. A second abrupt fall then ensued after a variable period, culminating in the death of the animal. Four to 5 minutes before death a failure of respiration occurred, and a minute or two later there occurred a sudden and intense constriction of all the muscular vessels (plate fig. 3) which resembled that seen in animals subjected to asphyxia.

Cutaneous. The marked ischemia, previously described, developed within

the first few minutes after the onset of bleeding and persisted until the death of the animals.

Mesenteric. In spite of the drastic fall in blood pressure there was no actual deterioration of the functional potentialities for maintaining the capillary circulation up to a few minutes before death. A slow trapping of blood from the active circulation occurred in the mesenteric capillary vessels. The pooled blood oscillated back and forth within the capillary bed and eventually accumulated in the venous end of the bed. This pooling and trapping created a mechanical obstacle to flow in the venules and small veins which made recovery increasingly difficult. Vasomotion was present throughout, although somewhat diminished in animals that went into circulatory failure. The reactions of the metarterioles, capillaries and venules to a blood-loss of 3.5 per cent are shown in plate figures 4, 5 and 6. Figure 6 shows especially well the trapping of blood in the venules and venous capillaries.

The most variable aspect of the response of the peripheral blood vessels to acute blood-loss was the degree to which an increased vasomotion developed and the duration of this hyper-activity. Of the 95 rats subjected to a blood-loss of 2.5 per cent and greater (see table 1) the large majority, 76 rats, showed a hyper-active vasomotion. In the remaining 19 augmentation of vasomotion was brief, lasting only 4 to 5 minutes. The loss of this was accompanied by the rapid development of an extremely poor venular flow.

In those rats in which the vasomotion showed a sustained hyper-active response, the capillary flow did not become disrupted until the blood pressure fell to 45 to 50 mm. Hg. On the other hand, in those rats in which the vasomotion showed a poor response, the capillary flow became disrupted at relatively high blood pressure levels (65-75 mm. Hg). The former group of rats lived on the average 55 to 60 minutes after the bleeding, while the latter lived only 30 to 40 minutes.

The small muscular venules (50-70 μ) may be considered as part of the capillary bed proper since their responses closely followed those of the other muscular components of the capillary bed. These venules, like the rest of the capillary bed and unlike the larger venules, took no part in the generalized vasoconstriction which follows acute hemorrhage involving a blood-loss of 2 per cent or more. This difference in response again emphasizes the independence of the reactions of the components of the capillary bed from the remainder of the vascular tree.

d. *Sequence in circulatory failure.* The sequence of events is illustrated by a summary of a typical protocol of a rat subjected to a blood-loss of 3.5 per cent of body weight. The features listed below, except for vasoconstriction, are limited to observations made on the blood vessels of the mesoappendix.

Vasoconstriction. When the blood-loss reached 2 per cent there was in the skin an over-all ischemia accompanied by an extreme narrowing of the arterioles and muscular venules which persisted throughout the syndrome. As the blood-loss approached 2.5 per cent, the vessels of the mesoappendix began to react, first the arteries (60 μ) constricting, then the larger veins, and finally the terminal portions of the vascular tree.

Vasomotion. As the blood-loss reached 3 per cent, the vasomotion increased. The ensuing restriction of the capillary flow was accompanied by a speeding up of the venular flow. This speeding up of venular flow was temporary. When the blood pressure fell below 50 to 60 mm., the flow became considerably slower. The vasomotion progressively diminished and, within 15 minutes after the slowing of the venular flow, the metarterioles lost their vasomotion and remained open. A continuous but slow flow from the metarterioles now developed and many of the capillaries became filled with stagnant blood.

Reduced outflow from bed. Only the most direct channels from arteriole to venule, the A-V-A, contained an active flow. The venules became filled with blood cells, a progressively larger proportion of which did not reach the larger venous vessels.

Backflow. Some of the blood in the larger venular branches, instead of continuing in the normal direction, began to exhibit a backflow into the venules and thence into the capillary bed.

Trapping. As the blood pressure fell below 50 to 55 mm. the stagnant condition involved more and more of the capillaries so as to include many of those in the arteriolar end of the bed. During the period of increasing stagnation the metarterioles gradually lost their vasomotion and remained partially dilated. The existing arteriolar flow was now diverted through shunts to the larger venules and the capillary bed proper was completely bypassed. As the arterial blood pressure fell below 40 mm., the backflow into the capillary bed became increasingly persistent, accentuating the accumulation of blood in the capillaries and venules. At the terminal stage the only flow to be noted was the backflow from the venules, which extended through the bed as far back as the arterioles. This was intermittent, and the intervening periods of stagnation progressively lengthened until death ensued, 49 minutes after the bleeding.

Text figure 2 is a composite word diagram of the sequence of changes in the peripheral blood vessels of the mesoappendix, and is based on observations of the 70 rats which died following acute blood-loss. The diagram separates the responses which are concerned with increased vasomotion (on right side of chart) from those which are a consequence of the hypotension and vasoconstriction of the larger blood vessels.

II. *Reactions to Stimuli Following Bleeding.* a. *Hyper-reactivity of visceral vessels to epinephrine.* In addition to the observational studies described in the previous section, tests were made to ascertain the responsiveness of the blood vessels in the mesoappendix to the topical application of epinephrine.

Attention was focussed on the arterioles, metarterioles, precapillary sphincters and muscular venules. As shown in our previous publication (2), these are the vessels of the capillary bed which are directly affected by the constrictor action of epinephrine. The true capillaries and non-muscular venules react only by changes in the rate of blood flow through them.

The "critical response" chosen was a temporary narrowing of the metarteriole sufficient to reduce but not stop the flow, figure 10. For the normal, unbled rat the concentration required of epinephrine in warm gelatin-Ringer's solu-

tion to exert this effect, to be regarded as the minimal effective concentration, was constant for a given rat but varied in different rats from about 1:1 million to 1:3 million. After the minimal effective concentration for a given rat had been determined on a selected metarteriole, the rat was bled and the test was then repeated at intervals throughout the syndrome. It was found that, a few minutes after any blood-loss of 1 per cent or greater of the body weight, the vessels became hyper-reactive to epinephrine and retained this response until shortly before the rat either recovered or succumbed.

The post-hemorrhagic tests with epinephrine were made in one of two ways: either by observing any change in the effect of applying the concentration used before bleeding, or by varying the concentration until one was found which produced the original "critical response."

In a typical case, a concentration of 1:2 million caused the "critical response" of a narrowing of the metarteriole and a slowing of the capillary flow which lasted 40 to 60 seconds. The rat was then bled 1 per cent of its body weight and, 2 minutes later, the same concentration of epinephrine produced, not only a complete occlusion of the metarteriole, but also a marked constriction of its feeding arteriole and a cessation of capillary flow which lasted 6 minutes.

In the second method of testing it was found that the minimal effective concentration for the bled rat was usually one-fourth, and sometimes only one-fifteenth, that required by the rat before bleeding. For example, in the majority of cases the minimal effective concentration for unbled rats was 1:2 million to 1:3 million, while for bled rats it was 1:5 million to 1:8 million. In several cases the hyper-reactivity was much higher, the minimal effective concentration dropping from 1:3 million for the unbled to 1:45 million for the bled rats.

The hyper-reactivity, when once established shortly after the onset of bleeding, persisted with no further change into the terminal stages of circulatory failure irrespective of the amount of blood-loss. The metarterioles maintained their hyper-reactivity until about 10 to 15 minutes before death, when the flow through them had become very sluggish and sporadic.

A peculiar feature, which developed during the earlier stages after hemorrhage, was a change in the reactivity of the vascular components of the bed relative to one another. Thus, the arterioles, which normally are less responsive than the metarterioles and precapillaries (cf 2), usually were found to be the first vessels to respond to the epinephrine.

Plate figures 7 to 11 are photographs of a region in the rat's mesoappendix illustrating the difference in action of epinephrine on several types of vessels before and after bleeding.

The vessels shown in figure 7 are an arteriole (25μ), a muscular venule (75μ) and a non-muscular venule leading into it in the normal state. Figures 8 and 10 show the response to epinephrine before bleeding.

Figures 9 and 11 show the response of the same vessels to the same concentration of epinephrine after bleeding.

For the latter test the region had been irrigated with gelatin-Ringer's solution to remove the epinephrine applied previously to restore the vessels to their orig-

inal state. The rat was then bled, via the tail, 2 per cent of its body weight. Two minutes after the bleeding a few drops of epinephrine (1:2 million) were applied and the photographs, figures 9 and 11, were taken immediately thereafter. By comparing figure 9 with figure 8 it will be seen that the muscular venule after bleeding did not show a response, as it did before bleeding. On the other hand, by comparing figure 11 with figure 10 it will be seen that the constrictor response of the arteriole is much more marked.

b. *Hyper-reactivity to mechanical stimuli.* This aspect was followed in four to six rats in each of the main bleeding categories. Evidence of this type of hyper-reactivity was obtained in both the mesenteric and cutaneous circulation. Stroking the surface of the interdigital web or of the mesoappendix with a blunt glass rod resulted in the immediate appearance of a hyperemic flare. This response was not abolished, regardless of the extent of circulatory failure, and could be induced to within a few minutes of death. It was particularly striking in the already ischemic capillary bed of the web during the early stage of hemorrhage.

The hyper-reactivity of the muscular vessels was also made evident by the reaction of the capillary bed in the interdigital web to occlusion of the femoral artery and vein by cuff compression of the thigh. Under normal conditions in the unbled rat, although the flow through the capillary bed was made completely stagnant by the cuff pressure, the vessels remained clearly visible because of the numerous blood cells within them. In contrast to this, it was noticed that, after a bleeding which was insufficient to produce complete ischemia, cuff occlusion resulted in a disappearance of all the capillary vessels. The venules, however, contained an increased amount of blood. Evidently, therefore, the difference in response to the arterial occlusion, viz., a blanching of the capillary bed in the bled animal and a non-blanching in the unbled animal, indicates an increased reactivity after bleeding, presumably of the smaller arteries and the arterioles.

DISCUSSION. Observations on the changes in the mesenteric capillary circulation resulting from acute hemorrhage in rats anesthetized with sodium pentobarbital are summarized in text figures 1 and 2. The changes are to be attributed not so much to a deterioration of the intrinsic reactivity of the components of the capillary bed as to a mechanical upset of the hemodynamic relationship responsible for normal flow.

Development of hyper-reactivity. The most significant compensatory reactions in the capillary bed are an enhancement of the vasomotion and a hyper-reactivity to epinephrine of the metarterioles and precapillaries. The enhancement of the vasomotion involves an increase in intensity of the constrictor phases and restricts the flow in the bed to the central channels. This continues as long as the arterial pressure is sufficient to sustain a unidirectional, though slowed, flow and to maintain the drainage mechanism of the bed. However, when the arterial pressure falls below 60 mm. Hg the blood flow through the bed becomes deficient. In rats anesthetized with sodium pentobarbital, the critical blood pressure range for maintaining a blood flow through the capillary bed lies between 60 and 40 mm. Hg. The hyper-reactivity persists throughout the syndrome. On the other hand, the augmented vasomotion now diminishes and exhibits a reversal in the

relative duration of its phases. The consequence of this is a spread of flow among the capillaries and further slowing of the blood flow through the bed. Conditions finally develop in which the changes in the capillary bed no longer are compensatory but, on the contrary, serve to antagonize the compensatory effects of the continued vasoconstriction elsewhere. When the arterial pressure approaches 40 mm., the effect of the deficiency of flow becomes so pronounced at the venous end of the bed that venous backflow develops. This causes a reversal of flow in the bed which may sometimes extend as far back as into the metarterioles.

The disturbance in the hemodynamics of the capillary circulation accentuates the congestion in the venous end of the capillary bed and progressively aggravates the general oligemia. A failure of the blood to return from the tissues into the veins finally marks the onset of the terminal phase of circulatory failure. The end result is stagnation throughout the entire capillary bed. It is significant that even at this late stage of approaching peripheral circulatory collapse the hyper-responsiveness of the muscular vessels of the bed to chemical and mechanical stimuli still persists.

The disorder in the capillary bed, therefore, is to be regarded as mechanical and not organic. It can be corrected by the infusion of a sufficient quantity of fluid. This fact and the high degree of responsiveness to chemical and mechanical stimuli maintained by the capillary bed indicate that the failure of the capillary circulation is not due to abnormalities inherent in its vascular components.

Earliest vasoconstriction in skin. It is well recognized that hemorrhage induces widespread vasoconstriction. That the minute vessels are included was observed in the skin of the ear and paw of cats and dogs by Mann (7) and Meek and Eyster (8). Page and Abell (9) observed similar effects in the vessels of the intestinal mesentery of the cat and dog, which included arteries and arterioles of 60 to 150 μ and veins of 100 to 250 μ in diameter.

The literature already contains references which indicate that the visceral and musculo-cutaneous vessels differ in the degree of their responsiveness to hemorrhage. Rous and Gilding (10) found that at comparable stages the vasoconstriction of the musculo-cutaneous vessels was generally more complete than that of the visceral vessels. Their conclusions were based on differences observed in bled animals in the distribution of injected dye-solutions and carbon-suspensions. Silfverskiöld (11) based a similar conclusion on his finding that bleeding caused a great reduction in the number of open capillary vessels in the skin and muscles, but not in the liver. Likewise, Sjöstrand (12) found that the minute vessels in the visceral organs were not appreciably reduced in caliber after a blood-loss as great as 20 to 50 per cent by volume and claimed that the pallor of the organs was due mostly to a constriction of the larger blood vessels.

Our observations, made simultaneously on the mesoappendix and the interdigital web of the foot of the rat, have shown that the cutaneous vessels always become constricted earlier than those of the mesoappendix and that the constriction of the vessels in the skin is more intense and remains for appreciably longer periods.

Vasomotion and hemodilution. The counterpart of vasoconstriction in the

capillary bed is the augmentation of the vasomotion of the metarterioles and pre-capillary sphincters, in which the constrictor phases appear more frequently and are more intensely developed than the dilator phases. This paper brings out the appearance of an accentuated vasomotion at the time when hemodilution is known to occur. Augmented vasomotion is a prominent feature of the recovery phase following moderate hemorrhage. The consequences are twofold. First, it restricts the flow to the central a-v channels so that the offshoots of these channels carry no arterial blood to the capillaries. Second, the active flow in the central channels serves to maintain an effective drainage of the inflowing branches coming from the capillaries. This favors a diffusion of fluid from the surrounding tissue spaces into capillaries. This mechanism sets up conditions which should favor the occurrence of hemodilution.

SUMMARY. A. *Blood pressure.* A significant drop of blood pressure in rats anesthetized with sodium pentobarbital occurs after a blood-loss of 1 per cent and more of their body weight.

B. *Mortality.* The maximum amount of acute blood loss than can be tolerated by the rat under nembutal anesthesia is about 3.5 per cent of its body weight.

C. *Vasoconstriction.* 1. In the skin, vasoconstriction always appears earlier, is more pronounced and is of longer duration than in the mesoappendix.

2. In the mesoappendix, the initial vasoconstriction is never sufficient to prevent a continuous, though slowed, flow and there is little or no stasis in the true capillaries. Continued vasoconstriction accompanied by drastic hypotension finally disrupts the capillary flow.

D. *Augmented vasomotion.* The augmentation of vasomotion during moderate, and during the early stages of severe, hemorrhage restricts the blood flow to the central channels of the capillary bed.

E. *Reactivity to epinephrine.* Hemorrhage induces, in the muscular vessels of the mesoappendix, a hyper-reactivity to the local application of epinephrine which continues into the terminal stage of the syndrome.

F. *Circulatory failure.* In the mesoappendix the flow from the arterioles tends increasingly to bypass the capillary bed through direct shunts into the venules. When the flow into the capillary bed fails, the blood, shunted across to the venules, reverses its direction in the venules and flows backward into the capillary bed, where it stagnates.

CONCLUSION

Acute hemorrhage accentuates certain normal features of the capillary circulation in the compensatory reactions against fluid loss. The main feature is an augmentation of the vasomotion, which contributes to hemodilution. The reactivity of the muscular components of the capillary bed to mechanical and chemical stimuli becomes hypernormal and persists into the terminal stages of the circulatory failure. It is to be inferred that the failure is due not so much to a deterioration of the intrinsic properties of the capillary bed as to the lack of propulsive force from the lowered blood pressure.

REFERENCES

- (1) ZWEIFACH, B. W. Anat. Rec. **73**: 475, 1939.
- (2) CHAMBERS, R. AND B. W. ZWEIFACH. Am. J. Anat. In press.
- (3) ZWEIFACH, B. W., R. E. LEE, C. HYMAN AND R. CHAMBERS. Ann. Surg. In press.
- (4) WIGGERS, C. J. Exper. Med. and Surgery **1**: 1, 1943.
- (5) CHAMBERS, R., B. W. ZWEIFACH AND B. E. LOWENSTEIN. This Journal **139**: 123, 1943.
- (6) DUNCAN, G. W., C. HYMAN AND E. L. CHAMBERS. J. Lab. and Clin. Med. **28**: 886, 1943.
- (7) MANN, F. C. Bull. Johns Hopkins Hosp. **25**: 205, 1914.
- (8) MEEK, W. J. AND J. A. E. EYSTER. This Journal **56**: 1, 1921.
- (9) PAGE, I. AND R. G. ABELL. J. Exper. Med. **77**: 215, 1943.
- (10) ROUS, P. AND H. P. GILDING. J. Exper. Med. **50**: 189, 1929.
- (11) SILFVERSKIÖLD, B. Skand. Arch. Physiol. **79**: 231, 1938.
- (12) SJÖRSTRAND, T. Skand. Arch. Physiol. **71**: 85, 1935.

TOTAL CIRCULATING PROTEIN AND HEMOGLOBIN IN THE GROWING RAT¹

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Hemoglobin (1, 2), plasma protein concentration (3, 4) and blood volume partitions (5, 6, 7, 8) vary with the stage of growth and size of the adequately nourished rat. Data which have been accumulated concerning blood or plasma constituents such as hemoglobin or plasma protein are usually expressed in terms of concentration, rather than total quantity; that is, as a two rather than a three-dimensional expression (9). It is probable that significant changes in the quantities of hemoglobin and plasma protein have been masked by measuring concentration without regard for the expanding or contracting blood volume, or without estimation of the total amounts of these substances present in the circulation (10, 11). Considering the relative magnitudes, specific variations in total amounts usually will become evident before concentration changes are apparent (5, 12, 13). The observed variations in the total circulating amounts of hemoglobin and plasma protein and their suggested relationship to rat growth constitute the basis of this report. The trend of these changes is similar to that occurring in humans at comparable stages corresponding to childhood (14, 15, 16), pubescence (15) and maturity (17, 18).

Five reports concerning total circulating hemoglobin and total circulating protein in the rat have been found in the available literature. Four of these are concerned with total circulating hemoglobin (19, 20, 21, 22); one, with total circulating protein (5). Each method required that the animal be sacrificed and, therefore, precluded a continued experiment. A relatively simple, comparatively accurate modification of the dye-dilution method which permits repeated determinations has been reported for estimating the blood volume partitions in the rat (6). Estimation of the total circulating hemoglobin (TCH) and total circulating protein (TCP) may readily be made with this method.

METHOD. Young, weanling (30 to 40 grams), male Sherman-strain rats were placed in individual cages and maintained on an adequate synthetic diet. This diet consisted of:

Sucrose	73 per cent
Casein (vitamin free).....	18 per cent
Corn oil (Mazola)	5 per cent
Phillips and Hart salt mixture IV	4 per cent

¹ Aided in part by a grant from the William W. Wellington Memorial Fund.

The water soluble vitamins² were added to the ration. The fat soluble vitamins³ were fed by syringe *per os* biweekly. Water was provided *ad lib*. The observed rate of growth was 3 to 4.5 grams per day. This may be considered to be a good increment of growth for this strain and diet. The animals appeared healthy throughout the experiment. Studies were done at three stages of growth using young (after 14 days on the experimental diet), pubescent (after 24 days on the experimental diet), and mature (31+ days on the experimental diet) rats. Hemoglobin, hematocrit and total plasma protein concentration determinations were made on thirty-four young animals. In twelve of these, blood and plasma volume partition determinations were also done. Similar determinations, including blood volume partitions, were made on nine pubescent rats not bled in the previous experiment. The hemoglobin, hematocrit and total plasma protein concentration determinations were repeated on twenty-five mature animals. Blood and plasma volume partitions were estimated in thirteen of these. All of the mature rats were from the previously bled young and pubescent groups. Two to 3 weeks were allowed to elapse in the interim between bleedings in every instance. This is ample time for both hemoglobin (21, 22, 23) and plasma protein regeneration (24).

Determinations were made on 0.8 cc. blood obtained from the ether-anesthetized animal by heart puncture. Dried heparin was used as the anticoagulant. Total circulating protein, total circulating hemoglobin and total mean corpuscular hemoglobin concentration (the ratio of circulating hemoglobin to circulating erythrocytes) were estimated only in those animals in which the blood and plasma volume partitions were concurrently measured.

PROCEDURE. Hemoglobin concentration was determined with the Klett-Summerson photoelectric colorimeter using a 540 $\mu\mu$ filter. Presence of the diazo blue dye T-1824⁴ in the plasma does not interfere appreciably with the hemoglobin determination, since the light absorption of the dye is maximal at 620 $\mu\mu$ (25).

The hematocrit may be calculated from the hemoglobin concentration since the mean corpuscular hemoglobin and mean corpuscular volume of rat erythrocytes are proportionally decreased (26). Moreover, changes in cell gravity usually parallel changes in cell hemoglobin, and the relationship is more or less constant (27, 28). Comparison of determined hemoglobins and hematocrits demonstrates the apparent relationship (12, 29). Cell volume concentration (hematocrit) may be estimated from the value for hemoglobin in grams/100 cc., where

$$\text{Hematocrit} = 2.95 \times \text{hemoglobin.}$$

The error is ± 0.4 per cent. The derivation of the formula has been discussed previously (6).

² Thiamine chloride 200 γ , Pyridoxine hydrochloride 200 γ , Choline chloride 100 mgm., Riboflavin 400 γ , Niacin 2500 γ , and Calcium pantothenate 1500 γ per 100 grams ration.

³ Haliver oil 0.5 gram, viosterol 0.25 gram, and α -tocopherol 0.375 gram made up to 37.5 cc. with corn oil, 0.05 cc./feeding.

⁴ Used in the blood volume procedure.

Total plasma protein concentration (grams/100 cc.) was determined gravimetrically by the copper sulfate method of Phillips, Van Slyke et al. (28). Since the partial specific volumes in terms of weight and nitrogen of animal and human plasma proteins are quite similar (30), the gravimetric method may reasonably be applied to estimation of plasma protein concentration in the rat without significant change in constants and involving only a small systematic error. Average specific gravity of adult rat plasma (twenty-five samples) was found to be 1.0302. This is somewhat higher than the value obtained for man (1.0264) (28). The specific gravity of pooled rat plasma samples determined by the copper sulfate method checked to within 0.1 per cent of the values obtained by the falling drop method used in the clinical laboratory.⁵ The gravity error of the copper sulfate method is ± 0.0002 , which entails an error of 0.06 cc./100 grams in the calculated plasma protein concentration. The dye, when diluted in the plasma, does not significantly alter the specific gravity.

Plasma volumes were determined by measuring the plasma dilution of a known amount of the injected blue dye T-1824. A single blood sample was obtained by heart puncture 3.5 to 4.25 minutes after dye injection. Concentration of the dye in the plasma was measured with the photoelectric colorimeter. Total plasma volume was calculated directly (6). To allow comparison, all volume measurements were adjusted to a unit of surface area (100 cm.²). Surface area was calculated by Lee's formula (31). Total circulating protein and total circulating hemoglobin were calculated by multiplying total protein and hemoglobin concentration by unit plasma and unit blood volume respectively. Total mean corpuscular hemoglobin concentration, the ratio of circulating hemoglobin to circulating erythrocytes, was estimated by dividing unit total circulating hemoglobin by unit total circulating cell volume.

OBSERVATIONS. The mean values for the data obtained are summarized in table 1. The "t-test" (32) has been used to compare means. The relation of the total quantities of hemoglobin and plasma protein in the circulation to the stage of growth is diagrammatically illustrated in figures 1 and 2. In this instance growth is expressed in terms of surface area.

Unit total cell volume apparently increases exponentially until maturity is attained;⁶ however, no significant increase in hemoglobin concentration (gram/100 cc.) is noted until after puberty in the growing rat. Thereafter, the increase is almost linear until maturity is reached.⁷ The values obtained for hemoglobin concentration in young and mature rats are similar to those noted by others (2, 4). In three-dimensional terms, the observed increase of unit total circulating hemoglobin⁸ is the resultant of increasing unit cell volume and hemoglobin

⁵ We are indebted to Dr. Otto Schales who performed the check determinations.

⁶ CV_u (pubescent vs. young) $t = 3.72$, $n = 19$, $P = < 0.01$; therefore significant. CV_u (pubescent vs. mature) $t = 2.31$, $n = 20$, $P = < 0.05$; therefore probably significant.

⁷ Hemoglobin (young vs. pubescent) $t = 1.48$, $n = 41$, $t = 0.1$; therefore not significant. Hemoglobin (pubescent vs. mature) $t = 5.06$, $n = 29$, $t = 0.01$; therefore significant.

⁸ Unit total circulating hemoglobin (pubescent vs. young) $t = 3.74$, $n = 19$, $P = < 0.01$; therefore significant. Unit total circulating hemoglobin (pubescent vs. mature) $t = 3.12$, $n = 20$, $P = < 0.01$; therefore significant.

concentration. The increase of hemoglobin mass appears to parallel the exponential body growth curve. The values obtained for unit total circulating hemoglobin are within the range of those previously found by the washing-out and carbon monoxide blood volume techniques (19, 20, 21, 22). By using the unit values for total circulating hemoglobin and total cell volume, mean corpuscular hemoglobin concentration assumes a total volume character. It is of interest that in rats, as in humans (29), the total mean corpuscular hemoglobin concen-

TABLE 1
Values at three stages of growth

	YOUNG	PUBESCENT	MATURE		YOUNG	PUBESCENT	MATURE
Weight (grams)	40.4-68.3 (34)	73.3-89.1 (9)	119.3-335.8 (25)	Unit blood volume (cc./100 cm. ³)	3.40 ±0.480	4.72 ±0.574	4.78 ±0.510
Surface area (cm. ²)	115.2-158.1 (34)	165.1-185.5 (9)	220.9-413.1 (25)	Unit cell volume (cc./100 cm. ³)	1.28 ±0.210	1.75 ±0.315	2.06 ±0.368
Hemoglobin (grams/100 cc.)	11.7 ±0.216 ±1.258 (34)	12.2 ±0.949 (9)	15.1 ±0.275 ±1.292 (22)	Blood volume (% body weight)	8.9 ±1.08	10.8 ±1.07	7.22 ±1.60
Hematocrit (cc. cells/100 cc.)	34.7 ±0.618 ±3.61 (34)	36.0 ±3.12 (9)	44.6 ±0.851 ±3.91 (22)	Circulating protein (grams/ PV_u)	0.135 ±0.0207	0.196 ±0.0219	0.217 ±0.0303
Total protein concentration (grams/100 cc.)	5.62 ±0.131 ±0.766 (34)	6.60 ±0.406 (9)	8.07 ±0.067 ±0.335 (25)	Circulating hemoglobin (grams/ BV_u)	0.429 ±0.0735	0.578 ±0.0871	0.711 ±0.105
Total blood volume (cc.)	4.75 ±0.91 (12)	8.67 ±1.07 (9)	14.21 ±2.48 (13)	Total mean corpuscular hemoglobin concentration (%)	33.5 ±0.99	33.3 ±2.08	34.8 ±2.73
Total plasma volume (cc.)	2.98 ±0.57 (12)	5.27 ±0.67 (9)	7.97 ±1.87 (14)				
Unit plasma volume (cc./100 cm. ³)	2.13 ±0.32	2.99 ±0.38	2.77 ±0.46				

Numbers in parentheses refer to number of observations contributing to mean and σ .

Note: Standard deviation of the mean (σ_{mean}) written after the mean;

Standard deviation of the samples ($\sigma_{\text{distribution}}$) written below the mean;

Where $n = (n - 1)$ in samples < 25 .

tration, like the mean corpuscular hemoglobin concentration, does not change with growth.⁹

A significant exponential increase in the total plasma protein concentration is noted at each of the three observed stages of rat growth.¹⁰ The values obtained

⁹ Total mean corpuscular hemoglobin concentration (mature vs. young) $t = 1.42$, $n = 22$, $P = < 0.2$; therefore not significant. Total mean corpuscular hemoglobin concentration (mature vs. pubescent) $t = 0.935$, $n = 20$, $P = < 0.4$; therefore not significant.

¹⁰ Total protein (young vs. pubescent) $t = 4.12$, $n = 41$, $P = < 0.01$; therefore significant. Total protein (pubescent vs. mature) $t = 10$, $n = 32$, $P = < 0.01$; therefore significant.

in young rats are in accord with those usually found at this age (4, 5, 33). The mean value obtained for mature rats is somewhat greater than that usually noted. If, as Whipple states, the plasma reflects the protein stores (34), it is possible that other stock diets were somewhat deficient in protein. It is more likely, however, that the discrepancy stems from the method used for analysis. In most reports, total protein concentration appears to have been determined by micro-Kjeldahl analysis. The conversion factor 6.25 is usually employed. Recently completed analyses of plasma from well-nourished rats demonstrates the factor to be 6.81,¹¹ which is in the middle of the range for humans (35). The difference could account for the discrepancy. The total circulating protein, calculated and ad-

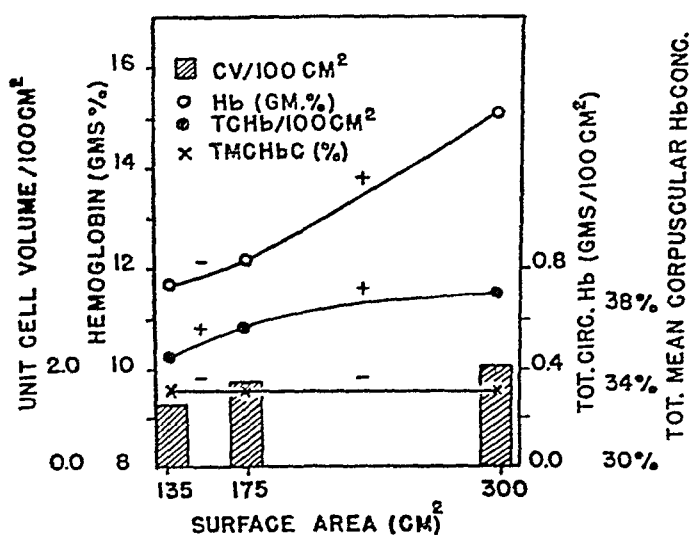


Fig. 1

Fig. 1. Hemoglobin and growth. The relationship and probable significance of changes in hemoglobin concentration, unit cell volume, unit total circulating hemoglobin and total mean corpuscular hemoglobin concentration with growth of the rat. Growth is expressed in terms of surface area. Plus sign indicates significant difference between dots.

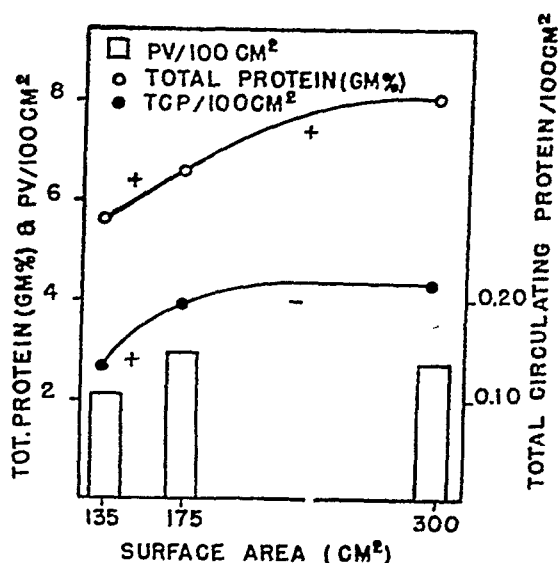


Fig. 2

Fig. 2. Plasma protein and growth. The relationship and probable significance of changes in total plasma protein concentration, unit plasma volume, and unit total circulating protein with growth. Growth is expressed in terms of surface area. Plus sign indicates significant difference between dots.

justed to unit volume, significantly increases during early growth. This increase is maximal at a time coincident with puberty. The slight observed increase with

¹¹ The specific nitrogen factor of the nondializable protein of whole plasma appears to be considerably greater than the conventional 6.25. The value observed is close to that determined for the nondializable proteins of normal human plasma, namely, between 6.6 and 7.0. As in the case of human plasma, the nitrogen factor will vary to a certain extent from pool to pool according to the bound lipid content of the beta globulins, and thus it may well be expected that in hypoproteinemic animals as in the case of hypoproteinemic humans a lower factor may be obtained.

complete maturity is not statistically significant.¹² The estimated mean value for unit total circulating protein in the mature rat differs from that obtained by Cutting and Cutter (5), but comparison is difficult. The similarities of the plasma volume and total protein concentration observed by both groups of workers suggest that the unit circulating protein value differences are dependent upon the relative difference in surface area as calculated.

The changes of plasma protein associated with growth are indicated in figure 2.

It is evident that all curves, with the exception of hemoglobin concentration and total mean corpuscular hemoglobin concentration, follow the exponential growth curve. The progression of the curves in each instance is somewhat different. Since no significant difference was found to exist between the mean values for total mean corpuscular hemoglobin concentration, it has been plotted as a straight line.

DISCUSSION. The values for the mass of hemoglobin and protein in the circulation presented in table 1 are *relative* rather than *absolute*. *Absolute* three-dimensional values for protein and hemoglobin would be largely dependent upon absolute accuracy of the method used to determine the blood volume partitions. Such accuracy has not yet been attained. Plasma dilution of the diazo blue dye T-1824 seemed to offer the best method, at the present time, for estimating these partitions in the rat with even relative accuracy. Two recent important criticisms of the dye method deserve mention. Relative to plasma volume, it has been suggested that dyes measure a fraction of the total plasma protein with which they combine rather than the actual plasma volume (36). Since the protein may be either in the blood or the interstitial spaces, this criticism is more significant when applied to extrapolation procedures. It is unlikely that a significant quantity of dye affixed to protein enters the lymph within 4 minutes in the rat. Relative to blood volume, use of the radioactive isotope of iron and viviperfusion in the dog has suggested an inherent systematic error when blood volume is estimated from the dye-determined plasma volume and venous hematocrit (37). The error would result in an apparently larger total cell volume. Four different variations, all employing the isotope, were used by Hahn et al. to determine the mass of erythrocytes. Each calculation, however, involved a venous hematocrit in the denominator. This is of some interest in that, as they conclude, the average hematocrit of the entire vascular system is considerably lower than that of the large vessels. Plasma volume was determined from a single sample obtained 4 minutes after dye injection. It has been stated that such an interval does not allow for complete mixing in the dog (38). Both of these discrepancies could conceivably result in the apparently smaller total cell volume which was observed. Since the mean total plasma volumes of the rat obtained by the dye method (6) are quite similar to those calculated from cell

¹² Unit total circulating protein (pubescent vs. young) $t = 7.14$, $n = 19$, $P = <0.01$; therefore significant.

Unit total circulating protein (pubescent vs. mature) $t = 1.74$, $n = 20$, $P = >0.1$; therefore not significant.

volume obtained by viviperfusion (5), presumably total cell volumes are similar in both instances. Were a systematic error inherent in the volume method used, the relationships to be discussed nevertheless would remain proportional.

Total plasma, cell and blood volumes are related to body size. This relationship is not linear. Unit plasma volume (cc./100 cm.³) attains its maximal value in the pubescent rat and maintains this level in maturity (6). Unit blood volume has a similar trend. It is interesting, however, that the blood volume expressed as per cent of body weight is significantly greater at pubescence than at any other time. Data on children suggest the transient occurrence of a similar event (15). This observation is not satisfactorily explained on the basis of physical size or age alone. It may be of some significance that the basal metabolic rate of pubescent rats is elevated at this time (39). The unit erythrocyte and hemoglobin mass appear to parallel body mass growth. The unit plasma volume grows more rapidly—attaining homeostasis at a body size coincident with puberty.

With these volume changes in mind, reference to figure 1 indicates that the three-dimensional measure of hemoglobin is probably more representative of the physiologic variation with growth. The apparent abrupt change in hemoglobin concentration becomes a gradual exponential change when the total amount of circulating hemoglobin is considered. The volume of hemoglobin normally contained in the blood is variable and appears to be influenced by growth. The volume of hemoglobin normally contained within the cell is less variable. Within the ranges observed, it is quite unchanging. Growth changes in unit circulating hemoglobin appear to parallel those of unit cell volume. If one calculates the hemoglobin from the hematocrit values recorded in the blood volume studies on children (14, 15) and adults (18), it is possible to determine the unit total circulating hemoglobin from the data. The trend of values obtained in such fashion is similar to that noted in rats.

Reference to figure 2 indicates that the homeostatic mechanism is not readily appreciated if plasma protein concentration—a two-dimensional measurement—is considered alone. It is more apparent if the unit total circulating protein is determined. As in the human (16), total plasma protein concentration increases with increasing maturity. The relative times at which plasma protein concentrations attain adult values, however, differ in the two species. In the human this value is reached between the first and second year of life (14); in the rat, shortly after puberty.¹² Growth changes in unit circulating protein apparently parallel those of unit plasma volume, and are maximal at puberty in rats. In man, maximal unit circulating protein values are apparently obtained before the end of the second year of life (14).

Amino acids derived from food provide the ultimate source of the construction materials needed in growth. These amino acids maintain a dynamic equilibrium between blood and tissues. Since muscle and plasma accept dietary protein nitrogen most readily, they represent the existing dynamic state most completely

¹² One hundred and nineteen to 122 grams growing rats have attained adult plasma protein concentration. In 6 such animals, the average total protein concentration was 8.0 gram per cent.

(40). Growth apparently depends in large part upon the early successful establishment of homeostasis between the extracellular fluid and the muscle and organ tissues. Homeostasis, in turn, is largely dependent upon relative constancy of any unit volume of the extracellular fluid (41). The relatively few observations of this experiment suggest that this mechanism is involved in the protein and hemoglobin economy of the growing rat. Total protein constancy is rapidly achieved in a unit volume of plasma while the surrounding body tissues continue to grow as the result of the dynamic equilibrium thus established. The apparent trend and relations of protein and of hemoglobin to the growth economy of the rat are in large measure duplicated in the human. In the latter species, however, relative plasma protein constancy is apparently attained at an earlier age.

SUMMARY. Forty-three weanling, growing rats were fed an adequate synthetic diet. The concentration of hemoglobin, erythrocytes and plasma proteins were determined in young and pubescent rats. Determinations were repeated in twenty-five of these animals after maturity was attained. Blood volume partition studies, using the dye T-1824, were done concurrently in most instances. These studies enabled calculation of the total circulating plasma protein and hemoglobin. The mean corpuscular hemoglobin concentration was calculated on a three-dimensional basis.

The plasma protein and hemoglobin content of the blood is better appreciated if the total circulating amounts are determined and adjusted to body size expressed in terms of unit surface area. The values so obtained are possibly more relative than absolute, but afford some insight into the dynamic adjustments occurring with growth in the rat.

CONCLUSIONS

The following conclusions are qualified by the relative smallness of the sample from which they are drawn:

1. Unit (cc./100 cm.²) blood and plasma volume attain their adult values coincident with pubescence.
2. Unit total cell volume increases exponentially until the rat reaches maturity.
3. No significant increase in hemoglobin concentration is noted until after puberty. The increase thereafter is almost linear. The adult value is attained coincident with maturity.
4. Unit total circulating hemoglobin gradually increases until the adult value is attained with maturity.
5. The total mean corpuscular hemoglobin concentration (ratio of circulating hemoglobin to circulating erythrocytes) does not change with growth.
6. Plasma protein concentration increases exponentially until the adult value is attained shortly after puberty.
7. The unit total circulating plasma protein is maximal at puberty and normally is relatively constant thereafter.

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8. These phenomena observed in the rat are similar to those reported for the human at comparable stages of development.

9. In the interpretation of quantitative changes of plasma protein and hemoglobin reference to total circulating amounts, rather than concentration, probably affords a better physiologic estimate.

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REFERENCES

- (1) GRIFFITH, J. Q. AND E. J. FARRIS. The rat in laboratory investigation. J. B. Lippincott Co., 1912.
- (2) ELLIS, L. N. AND O. A. BESSEY. This Journal **113**: 582, 1935.
- (3) DONALDSON, H. H. The rat. 2nd ed. Memoirs of Wistar Institute of Anatomy and Biology, no. 6, Philadelphia, 1924.
- (4) ALBANESE, A. A., L. E. HOLT, C. N. KAJDI AND J. E. FRANKSTON. J. Biol. Chem. **148**: 299, 1943.
- (5) CUTTING, W. C. AND R. D. CUTTER. This Journal **113**: 150, 1935.
- (6) METCOFF, J. AND C. B. FAVOUR. This Journal, **141**: 695, 1944.
- (7) CARTLAND, G. F. AND C. KOCH. This Journal **85**: 540, 1928.
- (8) GRIFFITH, J. Q. AND R. CAMPBELL. Proc. Soc. Exper. Biol. and Med. **36**: 38, 1937.
- (9) PETERS, J. P. J. Mount Sinai Hospital **9**: 127, 1942.
- (10) KEITH, N. M., L. G. ROWNTREE AND J. T. GERAGHTY. Arch. Int. Med. **16**: 547, 1915.
- (11) PETERS, J. P. AND D. D. VAN SLYKE. Quantitative clinical chemistry. Vol. I. Interpretations. Williams and Wilkins Co., Baltimore, 1937.
- (12) WEECH, A. A., M. WOLLSTEIN AND E. GOETTSCH. J. Clin. Investigation **16**: 719, 1937.
- (13) JANEWAY, C. A. New England J. Med. **229**: 779, 1943.
- (14) DARROW, D. C., H. C. SOULE AND T. E. BUCKMAN. J. Clin. Investigation **5**: 243, 1928.
- (15) BRINES, J. K., J. G. GIBSON, 2ND AND P. KUNKEL. J. Pediat. **18**: 447, 1941.
- (16) RAPOPORT, M., M. I. RUBIN AND D. CHAFFEE. J. Clin. Investigation **22**: 487, 1943.
- (17) ROWNTREE, L. G., G. E. BROWN AND G. M. ROTH. The volume of the blood and plasma. W. B. Saunders Co., Philadelphia, 1929.
- (18) GIBSON, J. G., 2ND AND W. A. EVANS. J. Clin. Investigation **16**: 317, 1937.
- (19) CHISHOLM, R. A. Quart. J. Exper. Physiol. **4**: 207, 1911.
- (20) BOYCOTT, A. E. AND R. A. CHISHOLM. J. Path. and Bact. **16**: 263, 1911.
- (21) SCOTT, J. M. D. AND J. BARCROFT. Biochem. J. **18**: 1, 1924.
- (22) BOYCOTT, A. E. J. Path. and Bact. **16**: 269, 1912.
- (23) PEARSON, P. R., C. A. ELVEJHEM AND E. B. HART. J. Biol. Chem. **119**: 749, 1937.
- (24) CUTTING, W. C. AND R. D. CUTTER. This Journal **114**: 201, 1936.
- (25) GIBSON, J. G., 2ND AND K. A. EVELYN. J. Clin. Investigation **17**: 153, 1938.
- (26) WINTROBE, M. M., H. B. SHUMACKER, JR. AND W. J. SCHMIDT. This Journal **114**: 502, 1936.
- (27) ASHWORTH, C. T. AND W. D. TIGERTT. J. Lab. and Clin. Med. **26**: 1545, 1941.
- (28) PHILLIPS, R. A., D. D. VAN SLYKE, V. P. DOLE, K. EMERSON, JR., P. B. HAMILTON AND R. M. ARCHIBALD. U. S. Naval Research Unit at the Rockefeller Institute for Medical Research, 1943.
- (29) WINTROBE, M. M. Clinical hematology. Lea and Febiger, Philadelphia, 1942.
- (30) COHN, L. J. AND J. T. EDSELL. Proteins, amino acids and peptides. p. 377. Reinhold Publishing Corp., New York, 1943.
- (31) LEE, M. O. This Journal **89**: 24, 1929.

- (32) FISHER, R. A. Statistical methods for research workers. 6th ed. Oliver and Boyd. London, 1936.
- (33) BERRYMAN, G. H. AND J. J. BALLMAN. This Journal **139**: 592, 1943.
- (34) MADDEN, S. C. AND G. H. WHIPPLE. Physiol. Rev. **20**: 194, 1940.
- (35) ARMSTRONG, S. H., JR. Personal communication. To be published with J. Metcalf.
- (36) COPE, O. AND F. D. MOORE. J. Clin. Investigation **23**: 241, 1944.
- (37) HAHN, P. F., J. F. ROSS, W. F. BALE, W. M. BALFOUR AND G. H. WHIPPLE. J. Exper. Med. **75**: 221, 1942.
- (38) GIBSON, J. G., 2ND, J. L. KEELEY AND M. PIJOAN. This Journal **121**: 800, 1938.
- (39) KIBLER, H. H. AND S. BRODY. J. Nutrition **24**: 461, 1942.
- (40) SHOENHEIMER, R. The dynamic state of body constituents. Harvard University Press, Cambridge, Mass., 1942.
- (41) GAMBLE, J. L. The extracellular fluid. Department of Pediatrics, Harvard Medical School, 1942.

THE MAINTENANCE OF A NORMAL SERUM CALCIUM BY THE PARATHYROID GLAND IN NEPHRECTOMIZED DOGS

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Although a definite influence of the parathyroid gland upon calcium metabolism has been known for many years, the mechanism of this effect has remained a disputed topic. A number of investigators have suggested that the action of the hormone on calcium metabolism is secondary to its effect upon phosphate excretion by the kidney (1, 2, 3).

The following work was undertaken to determine whether or not the gland could maintain the serum calcium level independent of kidney function.

METHODS. Large, adult dogs were used throughout the experiment. The average weight was about 20 kilos. All operative procedures were performed aseptically, and autopsy of all animals failed to reveal any infectious processes. In those animals in which the parathyroids were removed, the operation consisted of a total thyroparathyroidectomy. After operation the animals were allowed water *ad libitum* but were not given food or parenteral fluids.

Fifteen animals were bilaterally nephrectomized. In eight of these animals parathyroidectomy was carried out at the time of nephrectomy.

Samples of venous blood were secured from all animals immediately prior to operation. Subsequent samples were obtained 12 hours following operation, and at 24 hour intervals throughout the postoperative survival period. All blood samples were analyzed for serum calcium, serum inorganic phosphorus, alkaline serum phosphatase and non-protein nitrogen.

Serum calcium was analyzed by the method of Kramer and Tisdall (4) as modified by using the wash solution suggested by Wang (6), and precipitating the calcium oxalate for 4 hours. All determinations were done in duplicate and only those values which checked within 3 per cent or less, were accepted.

Serum inorganic phosphorus was determined by the method of Kuttner and Lichtenstein (7), adapted to the Klett-Summerson photoelectric colorimeter.

Phosphatase estimations were made by the method of Bodansky (8).

Non-protein nitrogen was determined by the method of Koch and McMeekin (9).

RESULTS. The average survival time of the animals undergoing thyroparathyroidectomy and nephrectomy was 74 hours; while the average survival time of the group having only the kidneys removed was 95 hours. Due to the acute terminal symptoms of tetany and uremia, only the values obtained for the 72 hour period following operation will be considered here.

The average serum calcium values of the nephrectomized animals remained within normal limits (11.0 to 10.2 mgm. per cent); while the serum calcium value of the thyroparathyroidectomized and nephrectomized group decreased from an

average control value of 11.3 mgm. per cent, to an average value of 5.9 mgm. per cent in an interval of 72 hours. See table 1.

The serum inorganic phosphate of both groups of animals was greatly elevated. The average value in 72 hours for the nephrectomized dogs was 25.6 mgm. per cent, an increase of 21 mgm. per cent above the average initial value. The animals with nephrectomy and parathyroidectomy averaged 17.1 mgm. per cent above the initial value. See table 1.

The alkaline serum phosphatase underwent a slow elevation following operation. There was no significant difference in this elevation between the two groups. In the 72 hour post-operative period the value for the nephrectomized group rose from an average of 1.4 to 3.6 Bodansky units. Similarly the control value of the thyroparathyroidectomized group was 1.2, which increased to 4.3 Bodansky units within the same period.

The non-protein nitrogen control value for the nephrectomized group was 29.7 mgm. per cent. This increased to an average of 322 mgm. per cent in 72

TABLE 1

Average serum values of nephrectomized-parathyroidectomized dogs as compared to a group of nephrectomized animals

POSTOPERATIVE HOURS	PARATHYROIDECTOMY AND NEPHRECTOMY					NEPHRECTOMY ONLY				
	No. of animals	Ca	P	Pt.*	N.P.N.	No. of animals	Ca	P	Pt.*	N.P.N.
Control.....	8	11.3	3.8	1.2	33	7	11.0	4.7	1.4	30
12 hours.....	8	10.4	8.3	2.6	60	7	11.6	9.5	1.9	59
24 hours.....	8	9.1	7.6	3.7	107	7	11.0	10.2	2.7	113
48 hours.....	7	6.7	11.4	5.2	196	7	10.7	13.8	2.7	207
72 hours.....	4	5.9	17.1	4.3	268	6	10.2	25.6	3.6	332

* Pt. = alkaline phosphatase in Bodansky units.

All other values in milligrams per cent.

hours. The control value for the thyroparathyroidectomized group was 33 mgm. per cent, which increased an average of 268 mgm. per cent in an equal period.

DISCUSSION. It has been suggested that parathyroid hormone exerts a direct effect upon the osteoclasts, thereby causing mobilization of calcium from osseous tissue (10, 11). The mechanism of such an action is, according to McLean and Bloom (12), simultaneous dissolving of both bone salts and organic matrix by a local cellular action.

Another belief has been that the hormone itself, or some substance formed by the hormone, is instrumental in increasing the solvent power of the plasma for calcium (13). This substance was thought to combine with the ionic calcium to form an organic compound. By this mechanism the concentration of ionic calcium in the plasma was lowered and that of the unionized diffusible organic calcium was increased. Since the concentration of ionic calcium of the plasma was thought to be in equilibrium with the calcium phosphorus complex of bone, this caused a mobilization of bone calcium into the plasma.

A concept that is receiving considerable current support is that the primary action of the parathyroids is upon the metabolism of phosphates (14, 15). It has been shown that the hormone increases urinary phosphorus excretion by reducing its reabsorption by the kidney tubules (16). The increased excretion of phosphorus reduces its concentration in the blood, and secondary to this decrease there is an increase in the calcium content of the blood (2, 3). In support of this view is the fact that extracts of parathyroid glands have no hypercalcemic effect when the kidneys have been removed or rendered nonfunctional (17, 18). Furthermore it has been observed by Neufeld and Collip that when a high concentration of phosphate is maintained by intravenous administration, it is impossible to produce a hypercalcemia with parathyroid extract (18).

These experiments (17, 18) show that a hypercalcemia cannot be produced by parathyroid hormone in the presence of a hyperphosphatemia, but furnish no adequate basis for concluding how the hormone influences the serum calcium concentration.

The results obtained in the present study show that the parathyroid gland can maintain a normal serum calcium level without kidney function, and independent of a wide variation in the concentration of inorganic phosphorus in the serum. The difference in the behavior of the serum calcium in the two groups of animals in spite of a marked elevation of serum inorganic phosphorus in both groups, is evidence for this fact.

SUMMARY

A decrease in the serum calcium concentration amounting to about 50 per cent in 72 hours was observed in nephrectomized-parathyroidectomized dogs. No similar decrease was observed in nephrectomized controls. The serum inorganic phosphorus and non-protein nitrogen were markedly elevated in both groups.

The evidence presented indicates that the parathyroid gland exerts an influence on calcium metabolism that is independent of the kidneys.

REFERENCES

- (1) SWINGLE, W. W. AND F. W. WERNER. *This Journal* 75: 372, 1926.
- (2) AUB, J. C., F. ALBRIGHT, W. BAUER AND E. ROSSMEISL. *J. Clin. Investigation* 11: 211, 1932.
- (3) BAUER, W., H. MARBLE AND D. CLAFLIN. *J. Clin. Investigation* 11: 47, 1932.
- (4) KRAMER, B. AND F. TISDALL. *J. Biol. Chem.* 47: 475, 1921.
- (5) CLARK, E. P. AND J. B. COLLIP. *J. Biol. Chem.* 63: 461, 1925.
- (6) WANG, C. C. *J. Biol. Chem.* 111: 443, 1935.
- (7) KUTTNER, T. AND L. LICHTENSTEIN. *J. Biol. Chem.* 86: 671, 1930.
- (8) BODANSKY, A. *J. Biol. Chem.* 101: 93, 1933.
- (9) KOCH, F. C. AND T. L. McMEERIN. *J. Am. Chem. Soc.* 46: 2066, 1924.
- (10) SELYE, H. *J. A. M. A.* 99: 108, 1932.
- (11) SELYE, H. *Endocrinology* 16: 547, 1932.
- (12) McLEAN, F. C. AND W. BLOOM. *Arch. Path.* 32: 315, 1941.
- (13) GREENWALD, I. *J. Biol. Chem.* 61: 33, 1924.
- (14) ALBRIGHT, F. AND R. ELLSWORTH. *J. Clin. Investigation* 7: 183, 1929.
- (15) ALBRIGHT, F. *J. A. M. A.* 117: 527, 1941.
- (16) HARRISON, H. E. AND H. C. HARRISON. *J. Clin. Investigation* 20: 47, 1941.
- (17) TWEEDY, W. R., R. D. TEMPLETON AND F. A. McJUNKIN. *This Journal* 115: 514, 1936.
- (18) NEUFELD, A. H. AND J. B. COLLIP. *Endocrinology* 30: 135, 1942.

THE SYNERGISTIC EFFECT OF CAFFEINE UPON HISTAMINE IN RELATION TO GASTRIC SECRETION

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It has recently been demonstrated that caffeine is a potent stimulant of gastric secretion in the cat and in man (1). In the course of these studies on the comparative effectiveness of histamine and caffeine as gastric stimulants, it was observed that after caffeine a subsequent dose of histamine provoked a greatly enhanced gastric secretory response when compared to the histamine response before caffeine. The purpose of the present investigation was to determine whether a synergistic relation between caffeine and histamine can be demonstrated, the term *synergism* being used to characterize the response obtained when the combined action of two drugs administered at the same time is greater than that which would be anticipated from the sum of their individual actions.

METHODS. *Cats.* Acute experiments on cats under light chloroform anesthesia were conducted using a modification (2) of the technique devised by Lim (3) which consists of ligation of the cardio-esophageal junction, exclusion of the vagi by previous dissection, and cannulation of the stomach with a perforated rubber tube inserted through the pylorus to permit continuous drainage of gastric juice. With cats prepared in this manner, the gastric secretory response to histamine, caffeine, and histamine plus caffeine was studied. The histamine used was the dihydrochloride (Imido, Hoffman-La Roche), injected subcutaneously; the dose varied from 0.2 to 0.5 mgm., but was kept constant for any given animal. An aqueous solution of caffeine with sodium benzoate was administered intravenously in a dose of 250 mgm. (containing approximately 125 mgm. caffeine base). In all experiments, before any stimulus was administered initially, the animals were allowed to become "basal"; *i. e.*, secreting no free acid.

The experiments on cats were divided into three groups as follows:

Group A. Histamine-caffeine-histamine sequence. In this group of 8 cats, histamine was injected subcutaneously and the gastric juice collected every 10 minutes for at least 70 minutes or until the response subsided and the basal level once again was obtained. Then, caffeine was given intravenously and after its stimulation had ceased, the same dose of histamine was again administered. The stomach was washed out with 20 cc. portions of distilled water at the cessation of stimulation as indicated by the volume flow from the cannula.

Group B. Caffeine superimposed on continuous histamine stimulation. The same dose of histamine was injected subcutaneously every 10 minutes throughout the entire course of the experiment. After the secretory response became constant at a plateau level for at least three 10 minute intervals, a single dose of

caffeine was administered intravenously. The histamine injections were continued for 1 to 2 hours after the superimposed effect of caffeine had subsided.

Group C. Histamine-caffeine-histamine plus caffeine sequence. In another group of 8 cats, the same procedure as outlined in (A) was used except that the third injection consisted of histamine (subcutaneously) plus caffeine (intravenously) simultaneously. The gastric juice was collected every 30 minutes for at least 3 hours after each stimulus was applied and the stomach washed twice with 20 cc. portions of distilled water after each interval. The washings were added to the secretions in making the titrations for free and total acid output.

Human Subjects. Fractional gastric analyses were conducted on 10 human subjects; all tests being performed after a fast of 10 to 12 hours. To obtain the "basal" level of secretion, the stomach was emptied completely of its contents every 10 minutes for a control period of one-half hour. Histamine was injected subcutaneously in doses varying from 0.05 to 0.2 mgm. in the different individuals, an attempt being made to use a dose giving a response approximately equal to that obtained from a constant dose of caffeine in previous experiments. The gastric contents were completely evacuated by aspiration every 10 minutes for a period of at least 70 minutes or until the "basal" level once again was obtained. Then, a caffeine test meal (200 cc. water containing 250 mgm. caffeine as the sodium benzoate) was introduced into the stomach via the Rehfuß tube. To permit exposure of the gastric mucosa to the test meal for a period of 30 minutes, a "block" technique was used as follows: For the next two 10-minute intervals, 10 cc. samples of gastric contents were removed for analysis. The purpose in removing these samples was to detect the first rise in acid concentration and thus the latent period. The stomach was emptied completely of its contents 30 minutes after the caffeine test meal was introduced and every 10 minutes thereafter for a period of at least 70 minutes or until the gastric secretion had returned to "basal" level. Having thus obtained in sequence the "basal" level of secretion and the secretory response to a control dose of histamine and a control caffeine test meal, both stimuli were applied simultaneously using the same doses of histamine and caffeine and the "block" procedure again conducted as outlined above.

Since alcohol presumably releases histamine by "irritation" of the gastrointestinal tract (4), another group of studies were made on 5 human subjects to determine whether or not a synergistic relation exists between caffeine and alcohol. A "block" procedure of fractional analysis was used throughout the experiment in the following sequence: "basal" control period, 50 cc. 7 per cent alcohol, 250 mgm. caffeine (as the sodium benzoate) in 50 cc. of water, and 250 mgm. caffeine in 50 cc. 7 per cent alcohol.

All samples of gastric juice in these experiments were titrated against 0.0274 N NaOH (1 cc. equals 1 mgm. HCl) for free and total acidity, using Töpfer's reagent (pH 3.5) and phenolphthalein (pH 8.5) respectively. In all cases the responses are expressed as the output of acid in milligrams of HCl.

RESULTS. *Cat.* The data in table I show that the subcutaneous injection of histamine in anesthetized cats after caffeine administered intravenously resulted

in a secretory response averaging 288 per cent (calculated on the mean response) greater than that obtained from the same dose of histamine given before the caffeine (an average change in the total output of free acid over a 70 minute period of 5.9 mgm. to 22.9 mgm. HCl). Cat 6 illustrates an observation made quite frequently in which a dose of histamine that failed to provoke a stimulation of gastric secretion before caffeine, did manifest an excitosecretory effect after caffeine. The duration of histamine response, as measured by the rate of flow from the cannula, was greatly prolonged after caffeine (an average change from 32 min. to more than 86 min.). It is to be noted that the gastric secretory effect of caffeine had subsided before the second injection of histamine was given. Control experiments in which the same dose of histamine was injected three times in sequence after returning to the "basal" level did not result in a greater response

TABLE 1

Gastric secretory response to histamine before and after caffeine in cats

CAT NO.	PRE-CAFFEINE SECRETORY RESPONSE TO HISTAMINE			SECRETORY RESPONSE TO CAFFEINE			POST-CAFFEINE SECRETORY RESPONSE TO HISTAMINE			PER CENT INCREASE
	Volume	Free acid	Duration of response	Volume	Free acid	Duration of response	Volume	Free acid	Duration of response	
	cc.	mgm. HCl	min.	cc.	mgm. HCl	min.	cc.	mgm. HCl	min.	
1	4.6	9.3	28	2.9	11.5	40	6.4	38.3	80+	312
2	5.2	10.6	30	3.1	12.6	34	7.5	22.5	95+	113
3	1.5	5.1	25	2.2	1.8	30	4.8	16.4	90+	221
4	2.0	2.0	22	5.2	22.1	40	6.3	12.8	90+	504
5	3.7	12.2	30	5.7	17.4	60	8.4	40.1	95+	229
6	1.2	0.0	34	2.7	4.5	40	3.2	13.0	70+	α
7	3.7	5.7	48	2.8	9.3	35	5.3	20.1	80+	253
8	2.1	2.1	37	5.5	15.8	47	7.0	19.8	90+	843
Ave.....	3.6	5.9	32	3.7	11.9	41	6.1	22.9	86+	353%

to the second or third injections; in fact, in some instances the total output of free acid became progressively less.

The results of experiments showing the effect of caffeine upon the continuous histamine secretory response are summarized in table 2. In this group of 4 cats, the same dose of histamine was injected subcutaneously every 10 minutes until a plateau level of secretion was maintained constant for 30 minutes at which time caffeine (250 mgm. as the sodium benzoate) was administered intravenously in a single dose; the histamine injections were continued for 1 to 2 hours after the effect of caffeine had subsided. In each case there was a marked and sudden increase in the volume and total output of free acid draining from the cannula. The plateau level of histamine secretion averaged 1.5 cc. and 5.9 mgm. HCl per 10 minutes; whereas the maximum average secretion per 10 minutes, when the effect of caffeine was superimposed upon the continuous histamine response was 2.6 cc. and 12.6 mgm. HCl. The average duration of increased gastric secretion

after caffeine was 75 minutes. The recovery continuous histamine secretory curve dipped below the pre-caffeine plateau and again rose to level off at a new somewhat lower plateau. This same phenomenon was observed with acetylcholine or acetyl-betamethylcholine superimposed upon continuous histamine stimulation (5).

In figure 1 the block graph represents the composite free acid response of 8 cats to histamine (subcutaneously); caffeine (intravenously) and histamine plus caffeine in sequence. The average total output of free acid for a period of 3 hours to histamine and caffeine given at the same time was 78 per cent (calculated on the mean responses) greater than the sum of the preceding individual responses to the same doses of histamine and caffeine given separately. From the graph it is evident that not only is the magnitude of stimulation enhanced but the duration of response is prolonged and maintained at a higher level. This type of

TABLE 2

*The effect of caffeine upon the continuous histamine secretory response in cats**

CAT NO.	BASAL SECRETION PER 10 MIN.			PRE-CAFFEINE CONTINUOUS HISTAMINE SECRETORY RESPONSE PER 10 MIN.			CAFFEINE + CONTINUOUS HIS- TAMINE SECRETORY RESPONSE PER 10 MIN. (MAXIMUM)				RECOVERY CONTINUOUS HISTAMINE SECRETORY RESPONSE PER 10 MIN.		
	Vol.	Free HCl	Total acid	Vol.	Free HCl	Total acid	Vol.	Free HCl	Total acid	Dura- tion	Vol.	Free HCl	Total acid
	cc.	mgm.	mgm.	cc.	mgm.	mgm.	cc.	mgm.	mgm.	min.	cc.	mgm.	mgm.
1	0.15	0.0	0.1	0.8	2.9	3.2	1.2	5.1	5.4	60	0.6	1.8	2.0
2	0.15	0.0	0.1	2.4	13.7	14.5	3.5	19.8	20.4	120	2.2	14.4	14.8
3	0.12	0.0	0.1	1.8	4.5	5.0	2.9	15.4	17.9	50	1.7	3.6	4.0
4	0.15	0.0	0.1	1.2	2.6	3.0	2.6	10.2	11.1	70	1.0	3.5	3.8
Ave....	0.14	0.0	0.1	1.55	5.9	6.4	2.6	12.6	13.7	75	1.36	5.8	6.1

* The dose of histamine di-hydrochloride every 10 minutes was 0.25, 0.4, 0.5 and 0.55 mgm. subcutaneously for the above four cats respectively. A single dose of 250 mgm. caffeine sodium benzoate was injected intravenously when the histamine response was constant for 30 minutes. Autopsy revealed diffuse multiple bleeding erosions of the gastric mucosa in every case.

secretory curve was obtained in each individual case. The average per cent increase calculated on the basis of the individual increments was 117 per cent (range 27 to 316).

Man. In figure 2 the composite secretory responses to histamine (subcutaneously), caffeine (by mouth) and histamine plus caffeine in 10 human subjects is presented in the form of a graph. The average total output of free acid for a period of 70 minutes to histamine and caffeine administered simultaneously was 65.3 per cent (calculated on the mean responses) greater than the sum of the preceding individual responses to the same doses of histamine and caffeine given separately for a similar period of time. Inasmuch as the duration of the response to the combined action of these drugs is prolonged and maintained at a high level, the degree of synergism manifested would be greater if the comparison were made for a longer period of time. This type of secretory curve was obtained in

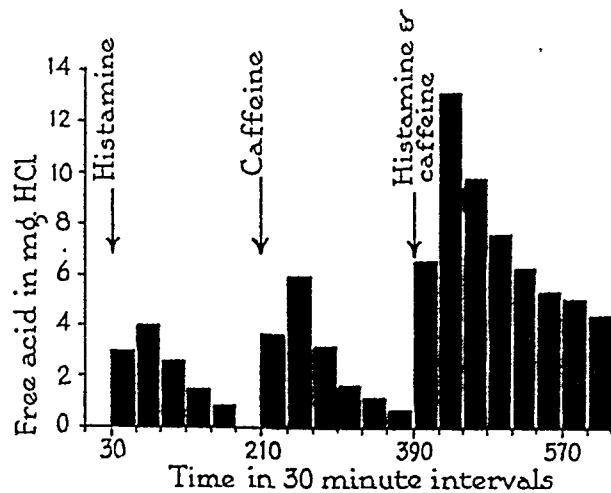


Fig. 1. Histograms showing the average total output of free acid in 8 cats to a sequence of injections: histamine di-hydrochloride (subcutaneously), caffeine with sodium benzoate (intravenously), and the same dose of both drugs simultaneously. The response to the combined action of histamine plus caffeine is not only of greater magnitude than the sum of the individual responses but also prolonged.

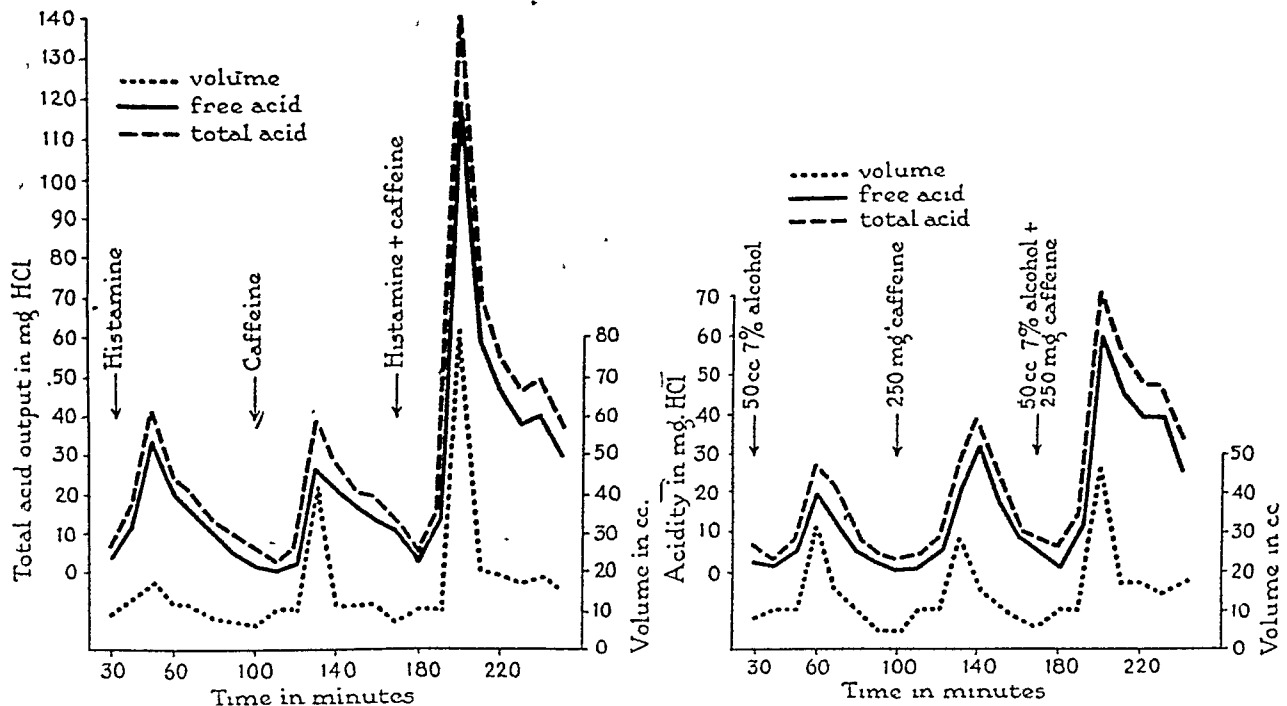


Fig. 2

Fig. 3

Fig. 2. Composite curves showing the average total gastric secretory response of 10 human subjects to the following sequence: histamine di-hydrochloride (subcutaneously), caffeine with sodium benzoate (via Rehfuß tube), and the same dose of both drugs simultaneously. The response to the combined action of histamine plus caffeine is not only of greater magnitude than the sum of the individual responses but also prolonged.

Fig. 3. Composite curves showing the average total gastric secretory response of 5 human subjects to the following sequence: 7 per cent alcohol (via Rehfuß tube), caffeine with sodium benzoate, and caffeine in 7 per cent alcohol. The response to the combined action of alcohol plus caffeine is not only of greater magnitude than the sum of the individual responses but also prolonged.

each individual case. The average per cent increase calculated on the basis of the individual increments was 153 per cent (range 7 to 760).

Similarly, as shown in figure 3, the response to the combined action of alcohol and caffeine given at the same time was on the average 65.9 per cent (calculated on the mean responses) greater than the sum of the individual responses to alcohol and caffeine given separately for a similar period of 70 minutes. This type of secretory curve was obtained in each individual case. The average per cent increase calculated on the basis of the individual increments was 76 per cent (range 33 to 173).

DISCUSSION. Necheles and his associates (5) have reported that acetylcholine or acetyl-beta-methylcholine and histamine were "synergists" relative to gastric secretion. Whether or not a true synergism, as defined above, exists between these drugs cannot be decided from the data they have presented inasmuch as a control experiment showing the response to each of the drugs separately was not performed.

With the type of experimental design used in this study, it has been possible to demonstrate a definite synergistic effect of caffeine upon histamine or alcohol in relation to gastric secretion. This synergism assumes practical importance in consideration of the ulcer patient. It is our opinion, based on our studies and the evidence in the literature, that the digestant action of acid and pepsin is an important factor in the pathogenesis, persistence and perforation of gastric or duodenal ulcers. The failure of atropine to abolish the high interdigestive secretion in duodenal ulcer has been taken as evidence that histamine or some histamine-like substance may be responsible for the secretion (6). A histamine cycle has been suggested in which the irritated mucosa liberates histamine which in turn stimulates gastric secretion; the action of the gastric juice on the ulcer liberates more histamine, and thus the cycle continues. The aggravating rôle which the consumption of caffeine-containing beverages might play in such a cycle in the ulcer patient is obvious. Also, as previously pointed out (2), caffeine given intravenously or by lavage to cats produces marked vasodilatation and tendency to hemorrhage in the mucosa and submucosa increasing their susceptibility to erosion and ulceration. If histamine is given repeatedly to provoke a continuous stimulation of gastric secretion in the cat for 10 to 12 hours, no apparent change occurs in the gastric mucosa. But, if a single dose of caffeine (125 mgm.) is superimposed upon this continuous histamine stimulation, diffuse desquamation of the mucosal epithelium in large areas and bleeding erosions and ulcerations appear in as short a time as 5 hours. This suggests that caffeine may provide a factor of "cellular toxicity" and hence lower the resistance of the mucosal cells to the ulcerative process.

The fact that the property of caffeine which enhances the response to histamine persists to manifest this effect after the stimulation of gastric secretion by caffeine has subsided is of considerable theoretical interest. It appears that the stimulation of gastric secretion may not necessarily be attributed to the same property of caffeine which is responsible for the synergism. The latter may be explained by several theoretical mechanisms: persistent increased blood flow

accompanying vasodilatation, alterations in cellular permeability, or an anti-histaminase rôle.

CONCLUSIONS

1. The gastric secretory response to histamine or alcohol is greatly enhanced and prolonged after caffeine in comparison to the output of hydrochloric acid or gastric juice provoked by the pre-caffeine administration of histamine or alcohol.

2. The gastric secretory response to histamine or alcohol and caffeine administered simultaneously is considerably greater than the sum of the preceding individual responses to the same doses of histamine or alcohol and caffeine given separately. The response to the combined action of these drugs is prolonged and maintained at a high level.

REFERENCES

- (1) ROTH, J. A. AND A. C. IVY. *This Journal*, **141**: 454, 1944.
- (2) ROTH, J. A. AND A. C. IVY. *Gastroenterology*, **2**: 274, 1944.
- (3) LIM, R. K. S. *Quart. J. Exper. Physiol.* **13**: 71, 1923.
- (4) DRAGSTEDT, C. A., J. S. GRAY, A. H. LAWTON AND M. RAMIREZ DE ARELLANO. *Proc. Soc. Exper. Biol. and Med.* **43**: 26, 1940.
- (5) NECHELES, H., W. G. MOTEL, J. KOSS AND F. NEUWELT. *Am. J. Digest. Dis.* **5**: 224, 1938.
- (6) IVY, A. C. *Surgery* **10**: 861, 1941.

INFLUENCE OF OXYTOCIN (PITOCIN) UPON THE HEART AND BLOOD PRESSURE OF THE CHICKEN, RABBIT, CAT, DOG AND TURTLE¹

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In 1912 Paton and Watson (1) reported that intravenous administration of posterior pituitary preparations caused a marked fall in the arterial pressure of the duck due to dilatation of the peripheral arterioles. They showed that this reduction in blood pressure was limited by a simultaneous increase in amplitude of the ventricular contractions though large doses (10 units) initially produced cardiac weakness. Gaddum (2) demonstrated that this depressor action was caused by the oxytocic fraction and was present in some cats as well as in the fowl. Additional proof that vasodilatation was responsible for the reduction in blood pressure was supplied by Morash and Gibbs (3) who showed that the oxytocic fraction lowered the blood pressure in the chicken. Coon (4) perfused isolated chicken hearts with Ringer Locke's solution and reported that pitocin increased coronary flow and strengthened cardiac contractions. Apparently, in the chicken, as in the duck, the mechanism of the fall in blood pressure is essentially vasodilatation. A reduction in the blood pressure and a marked dilatation of capillaries have also been reported in the frog (5, 6).

Recently (7, 8) it was shown that oxytocin (pitocin) lowered the arterial pressure in man but weakened instead of strengthened cardiac contractions. Some vasodilatation also occurred but this was not responsible for either the cardiac changes or the immediate reduction in the arterial pressure.

The differences in the mechanism of the fall of blood pressure in man as compared with the duck and chicken prompted these investigations upon the chicken, rabbit, cat, dog and turtle.

METHODS. Ether, morphine sulfate (8 to 20 mgm. per kgm.) or sodium phenobarbital (180 to 200 mgm. per kgm.) was administered forty to sixty minutes prior to the injections of the posterior pituitary preparations. Blood pressures were recorded optically by means of the hypodermic manometer (9) and control injections of pitressin either preceded or followed every injection of pitocin.

Studies also were made upon perfused isolated hearts. Oxygen from a tank was used to maintain the perfusion pressure of 100 mm. Hg. Ringer Locke's solution served satisfactorily as a perfusate for the rabbit and cat hearts, but not for the chicken hearts which deteriorated within a few minutes. However, satisfactory experiments upon the chicken hearts were obtained with a perfusate prepared as follows: $\text{NaCl}=6.1$, $\text{KCl}=1.58$, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}=0.43$, $\text{NaH}_2\text{PO}_4=0.25$, $\text{NaHCO}_2=1.6$ and $\text{CaCl}_2=0.29$ grams per liter adjusted to pH of 7.3-7.5

¹ Aid from a grant from Eli Lilly and Company is gratefully acknowledged. The Pitocin and pitressin used in this study were supplied by Parke, Davis and Company.

with 1 per cent NaOH. This solution conforms with the molecular concentration of ions in bird serum which markedly differ from those present in mammalian serum (10, 11). Ventricular fibrillation sometimes occurred in the chicken hearts. In the later experiments this tendency was reduced by maintaining the perfusate at a temperature of 34 to 35° C. Immediately before use, drugs were added to their respective bottles of perfusate which were kept in a constant temperature bath. In this manner the heart was perfused with known concentrations of drugs. During the experiments the source of the perfusate was controlled by means of glass stopcocks.

RESULTS AND DISCUSSION. *Chicken.* The intravenous injection of 0.1 oxytocic unit per kgm. of pitocin lowered the arterial pressure from 130/85 to 80/40. mm. Hg. within 7 to 10 seconds in five barbitalized, one etherized and one morphinized chicken. In every case the pulse contours showed the changes which are characteristic of arteriolar vasodilatation (8, 12) (see fig. 1). Evidence of cardiac weakness was not observed in any of the seven birds whether white leg-horns, plymouth rocks or white rocks were studied. The blood pressure and the pulse contours returned to the pre-injection normal within 20 to 90 seconds.

Similar but more prolonged effects were obtained following intravenous injections of doses as large as 50 oxytocic units per kgm. Little or no tachyphylaxis was apparent when injections were spaced 10 or more minutes apart.

Perfused heart experiments using the perfusate described above showed that 1 unit of pitocin per 10 cc. perfusate increased coronary flow 25 to 30 per cent but did not modify the amplitude of cardiac contractions. These observations on coronary flow are in agreement with those of Coon (4) though he found that pitocin strengthened cardiac contractions of the isolated heart. This is not in disagreement with the observation (13) that whole pituitary extract (which includes vasopressin) causes coronary vasoconstriction.

Rabbit. Among the eleven morphinized animals there were wide variations in the type and duration of the cardiovascular responses to the intravenous injections of pitocin. In three animals the blood pressure was unchanged or increased slightly. The oxytocic fraction evidently produced little or no effect in these three rabbits since similar effects were produced by the control injections of pitressin. In the other eight animals the blood pressure was transiently reduced, on the average from 130/95 to 50/43 mm. Hg, while the control injections of pitressin produced no change or a slight rise in blood pressure.

During the reduction in blood pressure the heart slowed markedly in one animal (fig. 2) and slightly in three animals. In two other animals (fig. 3) pulsus alternans developed. As shown below, cardiac slowing and alternate weak contractions also were produced by pitocin in isolated perfused hearts. Therefore these effects can be explained on the basis of the effect of the drug upon the heart itself.

With large doses of pitocin tachyphylaxis is pronounced in the rabbit even when an interval of 30 minutes separates the injections. As shown in figure 3, the first injection produces a greater response and it differs qualitatively from those produced by subsequent injections. The first large injection produced

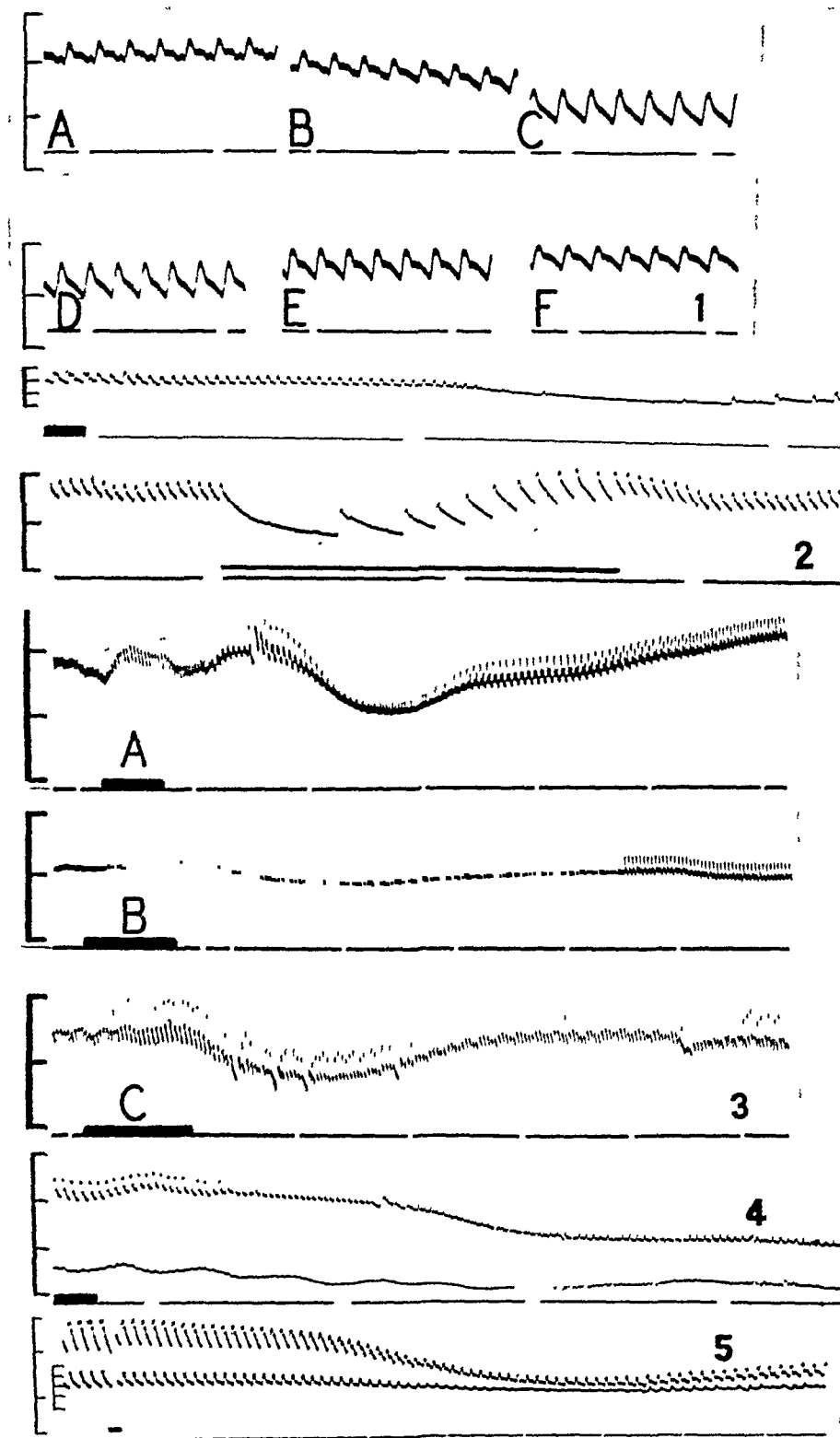


Fig. 1. Ischiadic arterial pressure pulses from chicken which had received 180 mgm./kgm. of sodium phenobarbital. In all records pressure scales are shown in units of 50 mm. Hg and base line is interrupted at 1 second intervals unless stated differently. *A*—control tracings; *B*—tracing obtained 5 seconds after intravenous injection of 0.15 oxytocic units/kgm. of pitocin; *C*—10 seconds after injection; *D*—30 seconds after injection; *E*—45 seconds after

changes in the pulse contours which are characteristic of weak cardiac contractions. In most but not all rabbits subsequent large injections administered after the elapse of a short interval produced pulse contour changes characteristic of vasodilatation. These observations indicate: 1, that in rabbits pitocin produces weak cardiac contractions and peripheral vasodilatation; 2, that tachyphylaxis of both effects occurs; and 3, that the tachyphylaxis of the cardiac effect is more pronounced and more persistent.

Evidence that vasodilatation generally plays only a minor rôle is supplied by oncometer tracings (fig. 4). During the period that the blood pressure is de-

injection; *F*—1 minute after injection. The contour changes which are characteristic of peripheral vasodilatation are very pronounced in tracing *C* obtained 10 seconds after the injection. Comparison of the pulse contours in tracing *A* with those of tracing *C* shows the following transient effects of pitocin: 1, the blood pressure in the chicken is markedly reduced; 2, at any given pressure the rate of pressure descent on the diastolic portion of the contour is increased; 3, the pulse contour becomes more central in type; 4, standing waves practically disappear. These effects which are characteristic of vasodilatation are very transient since one minute after the injection the blood pressure is returning toward normal and the pulse contour changes characteristic of vasodilatation are disappearing.

Fig. 2. Femoral arterial pressure pulses from rabbits which had received 8 mgm./kgm. morphine sulfate one hour earlier. The base line is interrupted at 10 second intervals. During signal in upper record 10 oxytocic units per kgm. of pitocin were injected intravenously. Fifteen seconds after the injection the blood pressure was markedly reduced, cardiac slowing was present and the small pulse pressure indicated that pronounced cardiac weakness was also present. In the lower record, during signal the left vagus was stimulated electrically. Vagal activity slows but does not weaken the cardiac contractions.

Fig. 3. Femoral arterial pressure pulses from rabbit which had received 10 mgm./kgm. morphine sulfate and 2 mgm./kgm. atropine sulfate one hour earlier. Tracings and the three injections of pitocin administered at signals were separated by an interval of 15 minutes between *A* and *B*, and 45 minutes between *B* and *C*. Base line is interrupted at 10 second intervals. The first injection *A* transiently reduced the blood pressure and pulse pressure and produced pulsus alternans. These changes can be ascribed to weakened cardiac contractions (see text). The second injection *B* and the third injection *C* lowered the blood pressure but caused little change in the pulse pressure and did not produce pulsus alternans. These changes can be ascribed to vasodilatation (see text).

Fig. 4. Carotid pressure pulses and intestinal oncometer volume changes of rabbit. The base line is interrupted at 10 second intervals. One hour earlier 10 mgm./kgm. morphine sulfate and 2 mgm./kgm. atropine sulfate were administered subcutaneously. At signal, 15 oxytocic units of pitocin per kgm. were administered. Within 25 seconds the blood pressure was markedly reduced and cardiac weakness became pronounced. The simultaneous decrease in intestinal volume was quite likely passive and caused by the reduction in blood pressure. However, during the next 10 seconds evidence of vasodilatation is present as indicated by increased intestinal volume in the presence of the sustained reduction in blood pressure.

Fig. 5. From above downward: carotid and femoral arterial pressure pulses from rabbit, and base line. At signal 5 oxytocic units of pitocin per kgm. were injected intravenously and 7 seconds of the record have been deleted. The arterial pressure is lowered, yet little if any evidence of vasodilation is present. The rate of pressure descent during diastole is not increased for any given pressure. Standing waves remain or even become more pronounced upon the carotid pressure pulses. Note the marked differences between the pulse contours in this figure and those in figure 1 where characteristic changes from vasodilatation are present.

clining the intestinal volume is decreasing. This is likely a passive change, but it does indicate that little or no vasodilatation has occurred. After the blood pressure is reduced, an increase in intestinal volume did appear and it is possible that some vasodilatation does occur (see above). However, pulse contour studies show that pitocin can lower the blood pressure in the rabbit by an action other than vasodilatation (see fig. 5). The initial reduction in arterial pressure and the small pulse pressure of usually 5 mm. Hg (figs. 2, 3, 4, 5) can be explained only by a marked reduction in the beat and minute output.

This decreased cardiac output in the presence of low arterial pressure could result either from decreased venous return or from feeble cardiac contractions. Both of these possibilities have been investigated. Pressures were recorded

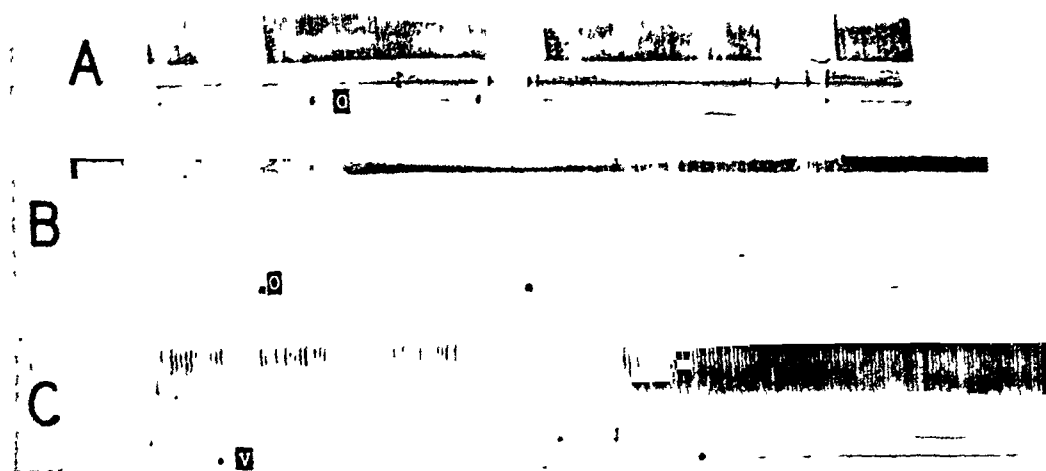


Fig. 6. Tracings obtained from isolated rabbit heart perfused with oxygenated Ringer-Locke's solution. From above downward: myograms, time line (interrupted at 1 sec. intervals in A and C, at 10 sec. intervals in B) and signal showing coronary flow in units of 10 cc. of perfusate. While recording A and C the speed of the kymograph was increased to show contour of one myogram during control period, during time of drug effect and after recovery from drug. Tracing B shows that the oxytocic fraction weakened cardiac contractions and may produce pulsus alternans by direct action while causing some coronary dilatation.

from the left and right ventricles of two morphinized rabbits. During the time that the systemic arterial pressure was reduced by pitocin the left and right ventricular diastolic pressures were increased 2 to 4 mm. Hg. This elevation of ventricular diastolic pressures proves that venous return to both ventricles was adequate to fill the ventricles. By elimination this leaves feeble cardiac contractions as the mechanism in the rabbit which is responsible for the immediate reduction in blood pressure by pitocin.

Additional proof is supplied by five perfused heart experiments. As shown in figure 6, the addition of small quantities of pitocin to the perfusate decreased the amplitude of cardiac contractions and in some cases caused alternate contractions to be excessively weak. This latter would correspond to the production of pulsus alternans in the intact animal. In one perfusion experiment, pito-

cin (0.1 unit per 10 cc.) caused sudden cardiac arrest which persisted thirty seconds after return to perfusion with Ringer Locke's solution. In two perfused heart experiments it was necessary to use 10 oxytocic units of pitocin per 100 cc. of perfusate to produce weak cardiac contractions. Tachyphylaxis was pronounced in the isolated perfused heart since the second time even less effect could be elicited with larger concentrations of pitocin. The reduction in blood pressure in the rabbit is apparently the result of weak cardiac contraction and a reduction in cardiac output as in man (7, 8), and the result of vasodilation as in the duck (1) and chicken (3).

Cat. In two barbitalized cats and in three of the four etherized cats, 15 to 20 oxytocic units per kgm. of pitocin lowered the arterial pressure from 120/75 to 80/50 mm. Hg for 15 to 30 seconds. These results are in agreement with those of Gaddum (2) who demonstrated that these large doses transiently reduced the blood pressure of some cats. During the reduction of blood pressure, definite evidence of vasodilatation could not be obtained. This may mean that the oxytocic fraction produces little or no vasodilatation in the cat. However, the pressor activity contaminant may be great enough in these large doses to mask any vasodilating activity of the oxytocin.

In three perfused heart experiments, cardiac contractions were weakened when the perfusate contained 10 oxytocic units of pitocin per 100 cc. In the cat as in the rabbit, pitocin weakens cardiac contractions.

Dog. In ten animals intravenous injections of doses as great as 10 oxytocic units of pitocin per kgm. produced blood pressure rises but no falls. These effects can be explained by the vasopressor content of the pitocin since similar changes were produced by the injection of an amount of pitressin which contained vasopressor activity equal to the vasopressor contaminant of the pitocin. These observations do not dispute the findings (14) that the oxytocic fraction may inhibit or abolish the typical depressor effect of the large doses of the pressor fraction. They do show that the oxytocic fraction does not lower the blood pressure in normal dogs. Unpublished data from this laboratory show that pitocin does lower the blood pressure of dogs which are pregnant or which have been pre-treated with stilbestrol.

Turtle. In six turtles 5 units of pitocin injected into the pulmonary artery or into the aorta supplying the body lowered the blood pressure in all three systems, the pulmonary artery, the aorta to the body and the aorta to the head region. All animals showed definite evidence of peripheral systemic vasodilatation in that during diastole at any given pressure the rate of pressure descent was increased. One turtle showed periods of more forceful cardiac contractions along with other periods of diminished contractions.

SUMMARY AND CONCLUSIONS

The oxytocic fraction of posterior pituitary preparations has cardiovascular actions. The species variations of these effects are pronounced. These changes are not those produced by the vasopressor principle and do not arise from a reduced coronary flow.

In the rabbit and cat intravenous administrations of pituitary preparations which contain the oxytocic fractions lower the arterial pressure and markedly weaken cardiac contractions as in man. Also vasodilatation often occurs in the rabbit and cat as in the chicken. In the turtle the transient reduction in the blood pressure arises mainly from peripheral vasodilatation as in the chicken. The oxytocic fraction does not lower the blood pressure in normal dogs.

REFERENCES

- (1) PATON, D. N. AND A. WATSON. *J. Physiol.* 44: 413, 1912.
- (2) GADDUM, J. H. *Ibid.* 65: 434, 1928.
- (3) MORASH, R. AND O. S. GIBBS. *J. Pharmacol. and Exper. Therap.* 37: 475, 1929.
- (4) COON, J. M. *Arch. Int. Pharmacodyn.* 52: 79, 1939.
- (5) DIETEL, F. G. *Arch. f. Exper. Path. u. Pharmacol.* 170: 417, 1933.
- (6) DIETEL, F. G. *Klin. Wehnschr.* 13: 554, 1934.
- (7) WOODBURY, R. A., W. F. HAMILTON AND P. P. VOLPITTO. *This Journal* 129: 500, 1940.
- (8) WOODBURY, R. A., W. F. HAMILTON, P. P. VOLPITTO, B. E. ABREU AND H. T. HARPER, JR. *J. Pharmacol. and Exper. Therap.*, 81: 95, 1944.
- (9) HAMILTON, W. F., R. A. WOODBURY AND H. T. HARPER, JR. *J.A.M.A.* 107: 853, 1936.
- (10) WINTERSTEIN, H. *Hand. der vergl. Physiol.* 1: 1117, 1925, Gustav Fischer, Jena.
- (11) CLARK, A. J. *Comparative physiology of heart.* P. 140, 1927, Cambridge Univ. Press, London.
- (12) HAMILTON, W. F. *This Journal* 141: 235, 1944.
- (13) ROSS, J. B., N. B. DREYER AND R. L. STEHLE. *J. Pharmacol. and Exper. Therap.* 38: 461, 1930.
- (14) MELVILLE, K. I. *J.A.M.A.* 106: 102, 1936.

THE SENSITIVITY OF THE RESPIRATORY CENTER TO HYDROGEN ION CONCENTRATION

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The sensitivity of the respiratory center to the hydrogen ion concentration, at one time accepted without doubt as a result of the work of Haldane, Hasselbach, Winterstein and Gesell, is very much in dispute now due to more recent work. The regulatory effect of CO_2 on the ventilation described by Haldane (1922) was attributed to its effect on changing the hydrogen ion concentration. The compensation of the acidosis due to fixed acids by increased ventilation and lowering of the CO_2 tension (Hasselbach, Winterstein) was explained by the sensitivity of the center to an increase in hydrogen ion concentration.

The discovery by Heymans and collaborators of chemoreceptor reflexes that could be stimulated not only by low oxygen tension but also by increased CO_2 and decreased pH brought up the question that the regulation might not be due to the effect of these changes on the center but to the chemoreceptor reflexes. As Schmidt and Comroe very clearly express it in their review (1940) the matter rests on the relative sensitivity of each mechanism. The question seems settled for the effect of anoxia and for the regulation by CO_2 . Anoxia produces stimulation of the chemoreceptors while it leads primarily to depression of the center (reviewed by Schmidt and Comroe, 1940, also Moyer and Beecher 1942, and Watt, Dumke and Comroe, 1943). The center on the contrary is much more sensitive than the chemoreceptors to changes in CO_2 tension (Schmidt and Comroe). As to the effect of hydrogen ion concentration Schmidt and Comroe still believed in that review that the center was much more sensitive to changes in pH than the receptors. The work of Nielsen (1936) introduced important contradictory evidence which seemed to indicate that the respiratory center is not sensitive to pH changes and that the regulatory effect of CO_2 is due to a specific action independent of its effect in changing the pH.

These results have confused the conception of the proper mechanism of stimulation of the respiratory center (Gesell, 1939), and have made Schmidt and Comroe reverse their opinion; in their own words (1941): "The status of the hydrogen ion as a stimulus to center and chemoreceptor is at present quite uncertain. The sensitivity of the center is apparently much lower and that of the chemoreceptors considerably higher than had been supposed. At the moment it seems quite possible that the respiratory effects of changes in pH, in so far as they are not referable to corresponding changes in carbon dioxide tension, are entirely due to chemoreceptor reflexes and not to a direct effect of hydrogen ions on the center, but this remains to be proved." According to Bernthal (1944), the whole problem is still open.

We have tried to simplify the controversy by eliminating one of the factors, namely, to study the effect of changes in hydrogen ion concentration not due to CO_2 , i.e., an acidosis induced by fixed acids in animals deprived of their chemoreceptor mechanism. If the acidosis is compensated by an increase in ventilation and a lowering of the CO_2 tension, the compensation must be due to a regulatory mechanism for the respiration which is sensitive to changes in pH. If compensation occurs without chemoreceptor mechanism we will be justified in accepting the respiratory center as the regulatory mechanism, and the accuracy of the compensation would give a measure of its sensitivity to changes in hydrogen ion concentration.

TECHNIQUE OF EXPERIMENTATION. *General procedure.* Dogs of 10 to 20 kilos weight were used. As soon as the necessary operations were completed the following data were determined: the minute volume ventilation and respiratory rate, the alveolar air CO_2 tension, the total CO_2 concentration of arterial blood. The CO_2 tension of the alveolar air was assumed to be a measure of the CO_2 tension of arterial blood as it has been repeatedly proved that the arterial blood is usually in equilibrium with the alveolar air as to the CO_2 tension (Bock and Field, 1924; Smith and Heinbecker, 1928; Bock *et al.*, 1929). From this CO_2 tension and the CO_2 concentration of arterial blood the pH of arterial blood was calculated. In one experiment the pH of arterial blood was also determined electrometrically by means of a glass electrode. These data obtained after completion of the operation and before the introduction of any acid were considered as the normal control values for the animal. While the analyses of the alveolar air and blood samples were being made acid was introduced intravenously at a very slow rate (0.5–1.0 cc. per min.). After the acid injection was completed an interval of 10 to 15 minutes was allowed for complete equilibration and compensation. Then a new set of data was determined. The procedure was repeated when possible with increasing amounts of acid injected.

Anesthesia. In most cases sodium barbital was used in doses of 350 mgm. per kilo when used alone and 300 mgm. per kilo preceded by morphia (2 early experiments). The barbital was injected intraperitoneally. In experiment 2 intravenous injection of nembutal was used in a dose of 33 mgm. per kilo. As the nembutal anesthesia is not very lasting, several succeeding doses of 65 mgm. had to be administered at irregular intervals. The level of anesthesia was very irregular and this type of anesthesia was discarded in later experiments. In experiment 6 the dog did not respond to the usual dose of barbital and as much as 450 mgm. per kilo had to be administered before complete anesthesia was obtained. The anesthesia level was, in this dog, unusually deep.

Operation. A tracheal cannula supplied with an additional narrow side tube was inserted. The femoral vein was used for acid injection while the femoral artery was used to obtain blood samples. The denervation operation was then carried out. It consisted of exposure of both carotid sinuses, ligation and section of both occipital arteries and the inter carotid nerves, cleaning the surfaces of the sinuses and of both the internal and external carotids for about one half inch from the bifurcation thus destroying the nerve network, and finally painting

the sinus and all the arteries of the region with 5 per cent phenol till the tissues turned to a whitish tinge. Both vagus nerves were cut in the mid cervical region.

Determinations. The main opening of the tracheal cannula was connected by means of a system containing Sudd valves and a three way cock to a calibrated recording respirometer. The dead space of the valve system was roughly equal to the dead space lost by the dog due to the fact that the cannula was placed about mid neck. The valve system had little resistance. It was left connected throughout the experiment. By a turn of the three way cock the expired air was collected in the respirometer. From the record obtained the minute volume and the respiratory rate were calculated.¹

The aveolar air was obtained by suction by means of an evacuated rubber bulb and a no. 10 French catheter. The catheter was introduced through the side tube of the tracheal cannula. The end of the catheter reached beyond the bronchial bifurcation into one of the smaller bronchi. The rubber bulb was supplied with a stopcock. It was evacuated by partial filling with water acidulated with sulfuric acid and subsequently expelling all the air and most of the water. The bulb was filled by momentarily opening the stopcock between respirations, i.e., after the end of one expiration and before the beginning of the next inspiration, and this repeated during a number of successive respirations. The mixed alveolar air sample thus obtained was analyzed for CO₂ per cent in a Haldane-Bayley gas analyzer. From the CO₂ per cent and barometric pressure at the moment (after subtraction of the water vapor tension at the dog's temperature), the CO₂ tension of the sample was calculated. Several bulb samples obtained under the same conditions gave results varying less than 1 mm. CO₂ tension. The value used was the average of 2 or 4 samples.

Arterial blood samples were obtained from the cannulated femoral artery. First about 10 cc. of blood were withdrawn to remove any possible stagnant blood; this was discarded. Then a sample was obtained under oil and immediately analyzed in the Van Slyke-Neill apparatus for the total CO₂ concentration of the arterial blood.

From the data the pH was calculated by means of the Henderson-Hasselbach formula using the values for CO₂ solubility coefficient and pK' given by Peters and Van Slyke (1937). In experiment 12 the pH was also determined electrometrically by means of a glass electrode, a Leeds and Northrop Thermionic amplifier and a Leeds and Northrop potentiometer reading to within 0.0001 volt. The glass electrode was contained in a completely enclosed electrode vessel. The blood was drawn directly from the artery by means of a hypodermic needle connected with the electrode vessel by a ground glass joint. To prevent the loss of CO₂ during the determination the blood was protected with heparinized saline which had been equilibrated with the dog's alveolar air obtained just before the blood sample. The so called "asymmetry potential," i.e., the combined

¹ As there was no great change of temperature or barometric pressure during the course of one experiment, and only relative changes in ventilation were considered, no attempt was made to correct the minute volume to standard conditions.

E.M.F. of the calomel cell, the glass electrode itself and the liquid junction, was determined just before each blood sample using 0.05 M potassium phthalate as the standard. The E.M.F. of the "asymmetry potential" in several determinations, as well as the E.M.F. of two consecutively drawn blood samples, agreed to within ± 0.0001 v. The pH determined electrometrically differed from the calculated pH by 0.03; this may be due to the fact that the pH was calculated for the blood at 38°C. while the electrometric determinations were made at 25°C.

Test of the effectiveness of the elimination of chemoreceptor reflexes. The most marked and most generally accepted function of the chemoreceptor reflexes is their response to low oxygen tension (see review by Schmidt and Comroe, 1940). In intact animals, breathing gas mixtures with low oxygen tension produces a reflex increase in ventilation. This increase in ventilation is replaced by the opposite effect, a decrease in the ventilation, when the chemoreceptor reflexes are eliminated. This phenomenon can be used as a test of the effectiveness of the chemoreceptors. Watt, Dumke and Comroe (1943) found that in animals deprived of chemoreceptor reflexes the depression of respiration due to breathing gas mixtures with low oxygen tension reaches a maximum after three to four minutes of breathing the mixture. Then the depression is followed by a stimulation. This delayed stimulation may disappear if the anesthesia is very deep (Moyer and Beecher, 1942).

In view of this we used the effect of breathing 10 per cent oxygen and 90 per cent nitrogen for three minutes as a test for the presence or absence of chemoreflex mechanism, comparing the effects on the minute volume ventilation before and after the denervation operation. This test was performed only in four of the animals of our series. In all four animals tested the original respiratory stimulation, i.e., increased minute volume ventilation, was replaced after denervation by a depression. The effect of breathing 10 per cent oxygen for three minutes was before denervation an increase in the minute volume ventilation of 28 per cent (expt. 9), 77 per cent (expt. 10), 16 per cent (expt. 11) and 43 per cent (expt. 12). After denervation the effect of breathing the same mixture for three minutes was on the contrary a decrease of 9 per cent (expt. 9), 10 per cent (expt. 11), 13 per cent (expt. 12) and permanent respiratory paralysis in experiment 10. As the operation was the same in all the animals used, and as the results of acid injection were similar in all the experiments, it is assumed that the chemoreceptor reflexes were not effective in any of the animals used.

RESULTS. The data from all the experiments are given in table 1. In every experiment where data could be obtained the results were similar. Gradual acid injection in dogs without chemoreceptor reflexes produced the following progressive changes:

a. The bicarbonate concentration of arterial blood decreased in all cases, and markedly when sufficient acid was added.

b. The minute volume ventilation increased. This increase was mostly due to an increase in depth of respiration although in some cases the rate was also increased.

TABLE 1

Effect of acid injection on dogs with the carotid sinus and bodies denervated and both vagi cut

EXPERIMENT NUMBER, WEIGHT OF DOG AND TYPE OF ANESTHESIA	ACID INJECTED		RESPIRATION		CO ₂ TEN- SION	ARTERIAL BLOOD					
	Type and concen- tration	Amount	Rate	Minute vol- ume		Total CO ₂	[H ₂ CO ₃]	[HCO ₃]	pH		
									Calcu- lated	Deviation from mean	Electro- metric
		cc.	per min.	liters	mm. Hg	mM	mM	mM			
1. 9.5 kilo. Morphine, barbital	0.5 N HCl	0	9	3.7	36.6	15.584	1.102	14.482	7.23	±0.000	
		20	10	4.4	30.0	12.969	0.903	12.066	7.23		
2. 14.0 kilo. Nembutal	0.5 N HCl	0	9	4.2	38.4	17.454	1.156	16.298	7.25	±0.050*	
		20	8	4.9	29.4	13.928	0.887	13.041	7.27		
		58	7	5.7	22.7	12.519	0.683	11.736	7.34		
		78	7	4.6	27.2	12.047	0.819	11.228	7.24		
4. 18.5 kilo. Morphine, barbital	0.5 N HCl	0	34	4.4	66.4	23.895	2.000	21.895	7.14	±0.005	
		35	31	5.1	63.8	22.410	1.920	20.490	7.13		
6. 15.0 kilo. Barbital alone— some in- trave- nously, some in- traperi- toneally	0.5 N HCl	0	9	3.0	35.8	20.471	1.078	19.393	7.35	±0.090*	
		50	9	3.1	36.4	18.810	1.095	17.715	7.31		
		72	9	3.1	32.9	18.743	0.990	17.773	7.35		
		122	12	5.0	21.1	16.074	0.635	15.439	7.49		
		157	13	5.8	23.6	14.715	0.710	14.005	7.40		
		220	11	7.5	15.6	8.370	0.470	7.900	7.33		
7. 20.0 kilo. Barbital alone	0.5 N HCl	0	10	3.4	41.9	20.894	1.261	19.633	7.29	±0.000	
		50	9	5.5	30.1	14.030	0.906	13.934	7.29		
8. 11.0 kilo. Barbital alone	1.0 N Lactic acid	0	5	1.8	87.6	23.445	1.132	23.313	7.40	±0.015	
		20	6	2.7	36.5	23.175	1.099	22.076	7.40		
		40	6	3.0	33.3	21.060	1.044	20.016	7.39		
		67	6	3.2	32.1	19.035	0.966	18.069	7.37		
9. 15.5 kilo. Barbital alone	0.5 N HCl	0	19	4.9	40.6	21.285	1.222	20.063	7.32	±0.025	
		30	19	5.0	38.5	19.466	1.159	18.307	7.30		
		75	23	7.2	30.7	16.435	0.924	15.510	7.32		
		105	24	8.3	25.2	14.201	0.759	13.448	7.35		
11. 10.5 kilo. Barbital alone	0.5 N HCl	0	29	4.6	35.2	18.171	1.060	17.111	7.35	±0.005	
		25	37	5.8	30.4	16.646	0.915	15.731	7.34		
		55	30	6.4	Dog died while samples were being taken						
12. 15.0 kilo. Barbital alone	0.5 N HCl	0	13	4.0	39.4	19.705	1.186	18.519	7.29	±0.005	7.253
		25	22	5.2	35.2	17.165	1.060	16.105	7.28		7.251
		70	24	6.5	26.8	13.110	0.807	12.303	7.29		7.251
		95	28	7.3	24.0	11.565	0.722	10.843	7.28		
Average deviation from mean.....										±0.008	±0.001

* Values not included in the average deviation; see text.

In experiments 3 and 5 the dog died during denervation operation.

In experiment 10 the dog died of respiratory paralysis while testing the effect of anoxia after denervation.

c. The alveolar air CO_2 tension, and therefore the arterial CO_2 tension decreased.

The changes in the bicarbonate concentration indicate the presence of a marked acidosis, yet this acidosis was compensated by a proportional drop in the carbonic acid concentration as indicated by the corresponding values for the pH of arterial blood. In some cases the compensation was very accurate throughout the experiment, the pH of arterial blood varying only a few hundredths of a pH. In two cases (expt. 2 and expt. 6) the compensation was not so accurate, the pH of arterial blood varying up or down in a greater degree (0.10 and 0.18 respectively). The greater variation in these two experiments might be due to the depth or the irregularity of anesthesia. It is indicative that it was found in experiment 2 where the dog had to be given repeated doses of nembutal as the anesthesia became insufficient, and in experiment 6 where the dog received as much as 450 mgm. of barbital per kilo. Discarding these two experiments on the ground that the dog was in a poor or variable state of anesthesia,² the average deviation from the mean in the pH of arterial blood during the course of the progressive acidosis amounts to $\pm \text{pH } 0.008$. In several cases the compensation was much more accurate, the pH changing less than 0.01, e.g., in experiment 7 an injection of 50 cc. 0.5 N HCl producing a decrease in bicarbonate concentration from 19.6 to 13.9 millimols without change in pH. The electrometric determinations made in experiment 12 although more accurate show an even smaller variation in the pH, injection of 70 cc. of acid producing a decrease in bicarbonate concentration from 18.5 to 12.3 millimols with only a change of pH of 0.002. This means that animals in this condition, i.e., anesthesia, ineffective chemoreceptor reflexes, are able to compensate a gradually increasing acidosis very accurately, at least to within $\pm \text{pH } 0.008$.

This compensation was obtained by increased ventilation with the consequent decrease in CO_2 tension. The effective stimulus to this increased ventilation, that is, the stimulus to the regulatory mechanism that brings about the compensation, cannot be the CO_2 . In all cases the ventilation increased further with a subsequent injection of acid even if the CO_2 tension was already much lower than the normal, for instance, in experiment 9 an addition of 30 cc. of 0.5 N HCl to the already injected 75 cc. increased the ventilation from 7.2 to 8.3 liters, while the CO_2 tension had been lowered to 30.7 mm. from the 40.6 mm. normal for this dog.

It is interesting to note, that in several of the experiments (fig. 1) the CO_2 tension and the minute volume ventilation during the progressively increased acidosis were in an inverse straight line relationship. The increased ventilation cannot be due to the CO_2 but, on the contrary, the CO_2 tension was a direct result of the ventilation.

DISCUSSION. Our results indicate that dogs deprived of chemoreceptor reflexes can compensate accurately for an acidosis produced by fixed acids (HCl or

² We are also justified in discarding these experiments from a statistical point of view, the deviation from the mean in them being more than four times the average deviation.

lactic) maintaining the pH of arterial blood at least within ± 0.008 . The compensation is due to a hyperventilation, with consequent lowering of the CO_2 tension and the carbonic acid concentration of arterial blood. In these cases of acidosis the effective stimulus to the ventilation cannot be the CO_2 tension as this was shown to be inversely proportional to the ventilation. We must seek another cause for the increased ventilation. As the ventilation seems to vary in such a manner as to maintain the constancy of the blood pH, it is logical to assume that the former is controlled by a mechanism which is sensitive to the hydrogen ion concentration of the blood. In these animals the known chemoreceptor reflexes were absent. Unless we assume the existence of other unknown

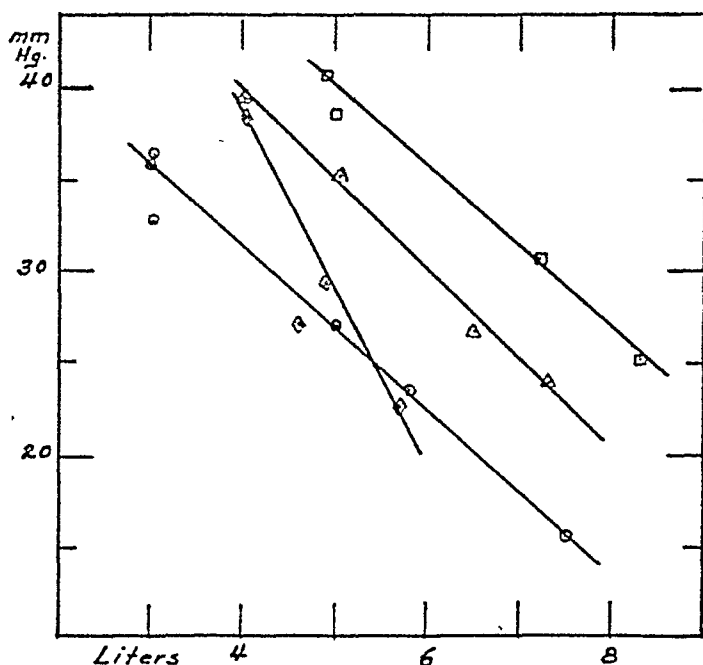


Fig. 1. The effect of successive intravenous injections of 0.5 N HCl on the respiration of animals with carotid sinus and bodies denervated and both vagus nerves cut. All determinations in four experiments.

Ordinate: Alveolar CO_2 tension in millimeters of mercury.

Abscissa: Minute volume ventilation in liters.

regulatory mechanisms not sensitive to low oxygen tension, we must accept the respiratory center as the regulatory mechanism. In order to regulate within certain limits of change the respiratory mechanisms must be sensitive at least to that degree of change, which in the present experiment was $\pm \text{pH } 0.008$. Assuming the accuracy of the electrometric determinations in experiment 12 the sensitivity may be even greater, the stimulus required amounting to as little as $\pm \text{pH } 0.001$.

Our results confirm the conception of Hasselbach, Haldane, Winterstein and Gesell, that the effective basic stimulus to the respiratory center is the hydrogen ion concentration. The effect of CO_2 could then be explained as due not to a mysterious specific effect but to its effect in changing the hydrogen ion concentra-

tion. It is interesting to note that the electrometric determinations of Experiment 12 indicate a sensitivity of the respiratory center to a change in pH (0.001) of the same order of magnitude as the change in pH that would be produced by the smallest change in CO₂ tension that affects the respiration. Haldane (1922) states that a change of CO₂ of 1.5 mm. (corresponding to 0.012 change in pH) suffices to double the ventilation and that one-twentieth of this change is sufficient to affect breathing. This corresponds to a change of pH of 0.0006.

There is of course contradictory evidence that has to be considered. Of this the most quoted among the modern results is that brought out by Nielsen. It is based exclusively on results of acidosis due to ammonium chloride ingestion and on two subjects only. In one of them a change in pH of 0.08 produced during the acidosis an increase of ventilation of 0.7 liter while in the same subject when not receiving ammonium chloride a change of pH of 0.04 due to CO₂ increased the ventilation by 10 liters. These results have been very much discussed from the point of view of possible equilibrium between the pH of blood and of the center (Gesell, 1939; Bernthal, 1944). In our opinion they should be analyzed from another point of view, the possibility of a depression of the respiratory center due to the ammonium chloride. Ammonium chloride acidosis is produced by the fact that the ammonia radical is converted to urea leaving the Cl radical to lower the bicarbonate concentration. Even small amounts of remaining ammonia might depress the respiratory center. This possibility has been overlooked because Nielsen postulates an increase in the irritability of the center during the ammonium chloride acidosis. Yet in our opinion his results indicate rather the opposite. In the first place his subjects show some tendency to compensate for the acidosis although incompletely. Their CO₂ tension dropped from a normal of 48 mm. to 36 mm. in one subject and from 44 mm. to 36 mm. in the other. Secondly his method of estimating the increase in irritability of the center, which he postulates to occur not only during acidosis but during anoxia and exercise as well, is based on a fallacious premise. He made his subjects breathe increasing amounts of CO₂ in the inspired air and determined the curve of the increase in ventilation plotted against the CO₂ tension. During acidosis as well as anoxia the curves are shifted to the left, i.e.: a greater ventilation is obtained at a lower CO₂ tension and on this fact he bases his argument for a greater irritability of the respiratory center. This is, in our opinion, taking the effect for the cause. Nielsen overlooks the fact that in his experimental conditions the ventilation could be, and certainly was, influenced not only by the CO₂ tension but by other factors as well, namely, in anoxia by chemoreceptor reflexes and in acidosis by the partial compensation as discussed above. It is well known now that during anoxia the center is actually depressed. A better index of the irritability of the center is in our opinion the slope of the curve, i.e., $\frac{\Delta \text{ ventilation}}{\Delta \text{ CO}_2 \text{ tension}}$. In at least one of his cases (his fig. 10) the slope of the curve is less during acidosis than in the normal individual, and in none of them is it greater.

Comroe (1943) argues against the sensitivity of the respiratory center to increased hydrogen ion concentration even when due to CO₂. He bases his con-

clusion on the frequent negative results of direct injection of acid solutions (lactic, hydrochloric, carbonic) into points of the medulla at the region of the respiratory center where stimulation of respiration could be produced either by bicarbonate and CO_2 mixtures buffered to pH 7.4, or electrically. We agree with his own statement in his discussion that the results cannot be considered very conclusive due to the artificial conditions; in our opinion the main point to be considered is the high acidity of the solutions injected into the nervous tissue. The least acid of his solutions (CO_2 in water at 70 mm. tension) had apparently a pH of 5.0. It has been known for a long time (Haldane, 1922) that increase in hydrogen ion concentration if great enough will depress and even stop respiration. In our own experience respiration ceases in dogs if the pH of the blood is maintained for a short time at around 6.8. The fact that a point that did not respond to acid stimulation could subsequently respond to electric stimulation is not conclusive either. He found a certain number of points that responded to electric stimulus and not to his own optimum chemical stimulus, namely, buffered CO_2 bicarbonate mixtures. One must also consider the possibility of a spread of the stimulating current.

Comroe found in the above quoted experiments not only points that responded to electric stimulation and did not respond to chemical stimulus, but also other points that responded to chemical but not to electrical stimulation. To this apparent dissociation of chemical and electrical responses may be added another fact. Comroe and Schmidt (1938) found that when the respiratory center of dogs is so depressed by anesthesia that it does not respond to such a strong chemical stimulus as inhalation of 10 per cent CO_2 , it still can respond reflexly. In those dogs ventilation was maintained by reflex stimulation from the chemoreceptors and ceased completely with pure O_2 inhalation or when the sinus nerves were cut. These facts bring out an interesting speculation.

One can postulate two separate systems in the respiratory center; one that originates impulses when the hydrogen-ion concentration increases within the cell and one that responds to impulses coming to it in an afferent reflex way. These two systems might be found in separate groups of cells with a final common path, and evidence for this may be derived from Comroe's results if confirmed. The two systems might just as well be postulated within the same cell; namely, the usual mechanism for responding to afferent reflex stimuli plus a system to respond to increased acidity. The latter could very well be an oxidation-reduction system that would easily be affected, as most biological oxidation-reduction systems, by anesthesia, O_2 lack, or increased acidity. The reflex system could remain effective when the oxidation-reduction system was ineffective, and both could act either independently or additively as to the number of impulses sent out by the cell. This conception if proved correct might eliminate a great deal of the confusion now existing as to the functions of the respiratory center. Some of the difficulties it might help to explain are, for instance, the reflex hyperventilation due to anoxia while the blood is more alkaline than normal and the center is depressed; it might also help to explain the so-called changes of irritability of the center due to reflex stimulation.

SUMMARY AND CONCLUSIONS

1. The ability to compensate for a gradually increasing acidosis due to intravenous injections of hydrochloric or lactic acids was tested in anesthetized dogs deprived of their chemoreceptor reflexes by denervation of the carotid sinus region and by section of both vagus nerves.

2. Test of the ineffectiveness of the chemoreceptor reflexes was made by finding that the usual hyperventilation due to breathing oxygen at low tension was replaced after denervation by a depression.

3. Results in all experiments were similar. The existing acidosis, as shown by the lowering of the bicarbonate concentration of arterial blood was compensated for by increased ventilation and a consequent lowering of the CO_2 tension of arterial blood, the latter being in inverse proportion to the volume of ventilation.

4. The compensation may be efficient enough to maintain the pH of arterial blood constant at least within ± 0.01 . As the chemoreceptors in the experimental animals were ineffective, we will have to accept the respiratory center as the regulatory mechanism unless we invoke some other unknown mechanism, and therefore the respiratory center must be sensitive to changes in hydrogen ion concentration at least as small as pH 0.01.

5. A speculative hypothesis is presented. It postulates the existence in the respiratory center of two mechanisms, one that would originate impulses under the stimulus of increased hydrogen ion concentration while a second would respond to afferent reflex impulses. These mechanisms might act both independently and additively and might be found either in the same or in separate cells.

REFERENCES

- BERNTHAL, T. *Ann. Rev. Physiol.* 6: 155, 1944.
BOCK, A. V., D. B. DILL, H. T. EDWARDS AND L. J. HENDERSON. *J. Physiol.* 68: 277, 1929.
BOCK, A. V. AND H. FIELD. *J. Biol. Chem.* 62: 269, 1924.
COMROE, J. H., JR. *This Journal* 139: 490, 1943.
COMROE, J. H., JR. AND C. F. SCHMIDT. *This Journal* 121: 75, 1938.
GESELL, R. *Ann. Rev. Physiol.* 1: 185, 1939.
HALDANE, J. S. *Respiration*. New Haven, 1922.
MOYER, C. A. AND H. K. BEECHER. *This Journal* 136: 13, 1942.
NIELSEN, M. *Skand. Arch. f. Physiol.* 74: sup. 10, 87, 1936.
PETERS, J. P. AND D. D. VAN SLYKE. *Quantitative chemical analysis*. Baltimore, 1937.
SCHMIDT, C. F. AND J. H. COMROE, JR. *Physiol. Rev.* 20: 115, 1940.
Ann. Rev. Physiol. 3: 151, 1941.
SMITH, R. J. AND P. HEINBECKER. *This Journal* 84: 271, 1928.
WATT, J. G., P. R. DUMKE AND J. H. COMROE, JR. *This Journal* 138: 610, 1943.

ACID-HUMORAL INTERMEDIATION IN THE RECTUS ABDOMINIS OF THE FROG¹

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Among the miscellaneous systems classified as cholinergic (Dale, 1936) striated muscle seems to offer special opportunities for studying the acid humoral mechanism of stimulation. Its innervation is sparse, usually only one or two motor end plates to a single fiber (Kuffler, 1941) and its stimulation by extraneous acetylcholine is known to occur through the intermediation of the motor end plates (Buchthal and Linhard, 1937). Humoral stimulation by extraneous acetylcholine appears to be of the simplest nature conceivable for each motor end plate acts as an independent source of stimulation to the muscle fiber whether that fiber be provided with one or more motor end plates. Unlike the neuron, apparently organized to sum its abundant and crowded synaptic potentials into one *common* electrotonic current initiating the impulse at the *distant* axon hillock (Gesell, 1940), muscle is thought to be stimulated *locally* by each motor end plate potential in the manner illustrated in figure 2. The contractions so initiated travel independently in opposite directions and are automatically extinguished on reaching the end of the muscle fiber or on collision with a similar approaching wave of contraction (Brown, 1941; Kuffler, 1941).

The strength of a composite contraction of a muscle submerged in acetylcholine is no doubt determined by the number of muscle fibers which are stimulated and by the frequency of their twitches, just as the strength of a reflex contraction is determined. With these points in mind we have endeavored to determine whether acid favors humoral intermediation in striated muscle as it was found to do in the central nervous system, and the heart (Gesell, Brassfield and Hamilton, 1942; Gesell, Brassfield and Hansen, 1942; and Brassfield and Gesell, 1942).

METHOD. The rectus abdominis of the frog, commonly employed as an indicator of acetylcholine, is prepared and mounted on a specially constructed frame and subjected to acid-modified acetylcholine-containing Ringer's solution. The experimental procedures are similar to those employed in studying the acid humoral control of heart beat (Gesell, Mason and Brassfield, 1944). Although the rectus abdominis was used entirely to study the acid-humoral intermediation of stimulation, the long parallel fibered sartorius muscle was found particularly suited to reveal the mechanisms of humoral recruitment. We have accordingly included one introductory observation on this muscle.

RESULTS. *Response of the sartorius muscle to graded immersion in acetylcholine solution.* Buchthal and Linhard (1937) showed that localized application of

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acetylcholine to a single motor end plate produces "short, rapid, tetanic contractions" of the corresponding muscle fiber. "Application of acetylcholine to other points on the fiber directly, in three to ten times higher concentrations than threshold for the end plates, has no effect." In agreement with these most significant findings establishing the intermediary function of the motor end plate we have found that a gradual immersion of an extended sartorius muscle into a weak solution of acetylcholine produces no visible contractions until that portion of the muscle provided with motor end plates is submerged. Once this level is reached the strength of contraction increases with the submergence. If the submergence occurs in large, abrupt steps, successively exposing new groups of end plates to the action of acetylcholine, the contraction builds up in a step-like manner indicating that each increase in strength of contraction is caused by the



Fig. 1

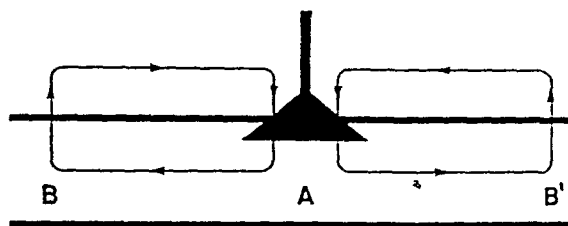


Fig. 2

Fig. 1. Response of the sartorius muscle of the frog to step-like immersion in acetylcholine solution.

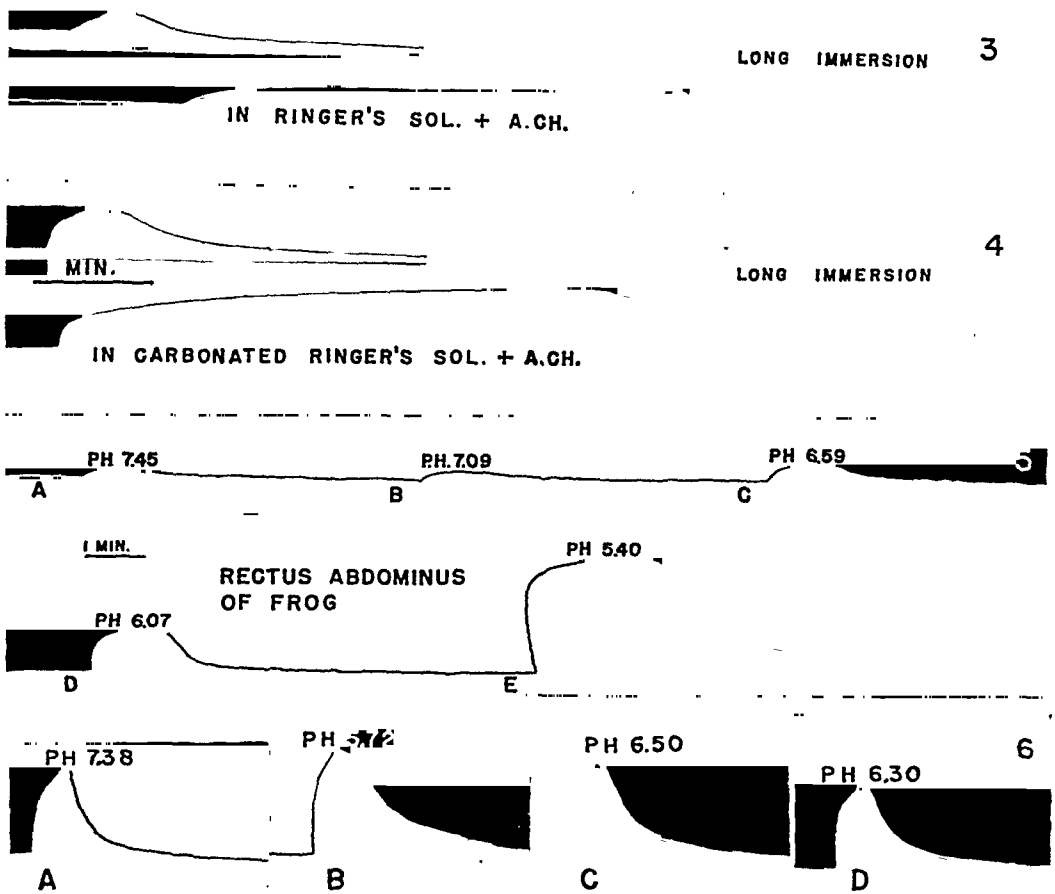
Fig. 2. A schematic representation of electrotonic stimulation of a muscle fiber. A local liberation of acetylcholine at the motor end plate *A*, like a droplet of chloroform on *Nitella*, sets up two circulating currents which stimulate at the outflow points *B* and *B'*. If the current is very transitory only a single response may occur but if prolonged the response will be rhythmical.

activation of additional muscle units (see fig. 1). Recruitment of this nature will be seen to play an important rôle in the interpretation of the results described in the following sections.

Response of the rectus abdominis to short and long exposures to non-carbonated and carbonated acetylcholine solutions. Carbonation is attained by bubbling the solution with carbon dioxide. Long exposures of the rectus abdominis to acetylcholine solutions, whether carbonated or not, produce higher contractions and more prolonged periods of relaxation (i.e., after-contraction) than do shorter exposures (figs. 3 and 4). In figure 3 in which a non-carbonated acetylcholine solution is used, an exposure of 4.5 minutes produces a tracing 6.3 mm. high as compared with 4.5 mm. in the shorter exposure of 30 seconds, an increase of 25 per cent. In figure 4 in which the acetylcholine solution is carbonated the augmenting effects of long exposure are proportionately much greater. The tracing obtained with long exposure is 14.5 mm. high as compared with 6.5 mm. in the tracing obtained with the short exposure, representing an increase of 123 per cent. The greater heights of contraction in the prolonged exposures are the result of a long sustained and gradual shortening of the muscle which is super-

imposed upon the short, rapid, introductory contraction. Prolonged contractions may, therefore, be divided into the first and second phases of shortening.

Granting that the strength of muscular contraction is primarily a function of the number of fibers that are activated, the pattern of recruitment of new muscle units must determine the pattern of the muscle tracing. Superficial muscle fibers are no doubt reached by the acetylcholine within a relatively short interval of time following the submersion of the muscle as compared with the considerable time required for the acetylcholine to reach the underlying fibers, even in a thin flat muscle such as the rectus abdominis. The depth of penetration of the



Figs. 3-6

acetylcholine is determined by the concentration of the acetylcholine in the surrounding solution, the velocity of its diffusion, the rate of its destruction by the cholinesterase of the muscle tissue, and the duration of the submergence of the muscle. Each of these factors plays a part in determining the total number of muscle fibers activated. It, therefore, seems likely that the first rapid phase of shortening is the result of a sudden and almost simultaneous activation of the outer muscle fibers readily reached by the acetylcholine and that the second long continuing phase of contraction is an effect of a progressive and more gradual recruitment of the deeper and more inaccessible fibers.

The contractions produced by the carbonated acetylcholine solutions, whether immersion be long or short, are considerably greater than those produced by non-carbonated solutions. In the shorter exposures, figures 3 and 4, carbon dioxide increased the height of tracing from 4.5 mm. to 6.5 mm. or 44 per cent; in the longer exposures from 6.3 mm. to 14.5 mm. or 132 per cent.

The speed of shortening is also affected by carbon dioxide. This is best seen in the slow secondary contraction. In the prolonged submersion in a non-carbonated acetylcholine solution, figure 3, it is considerably slower than in a carbonated solution, figure 4. This suggests that acetylcholine entering muscle without the protection of carbon dioxide undergoes more rapid destruction. The greater speed and strength of contraction of muscles submerged in carbonated acetylcholine solution indicates a deeper and more rapid penetration of acetylcholine and a greater recruitment of muscle fibers.

It seems probable that the greater intensity of contraction of a muscle submerged in carbonated acetylcholine solution is due to a greater frequency of twitch as well as to a greater recruitment of muscle fibers, for a readier penetration of acetylcholine under the protective action of carbon dioxide must cause higher concentrations of acetylcholine in the muscle. This no doubt holds for both short and long submersions. Granting that higher concentrations of acetylcholine set up more powerful electrotonic currents of stimulation at the motor end plates, high twitch frequencies and greater strength of contraction of the muscle are to be anticipated.

Carbonation of acetylcholine solutions not only increases the strength of contraction but prolongs the periods of relaxation (i.e., the residual contraction which is recorded after the muscle has been removed from the acetylcholine-containing solutions). This effect is similar to that produced by a prolongation of submersion of a muscle in an acetylcholine solution. Carbonation increased the duration of the "after-contraction" from 50 to 280 seconds in the short immersion of figures 3 and 4. In the longer immersion it increased the "after-contraction" from 130 to 420 seconds. These analogous effects of increased acidity of the muscle and of increased duration of exposure of the muscle to acetylcholine are conceivably a common function of the increased amount of the acetylcholine harbored by the muscle at the end of the period of submergence in the acetylcholine containing solution. Since both prolongation of immersion and acidification of the muscle should increase the amount of acetylcholine harbored by the muscle their individual effects should be additive. This would explain the great shortening of muscle occurring during a prolonged submersion in a carbonated acetylcholine solution as well as the prolongation of the period of after contraction. The muscle exposed to a carbonated acetylcholine solution would not only accumulate greater amounts and greater concentrations of acetylcholine but the greater acidity attained would protect this harbored acetylcholine and extend the period of residual contraction.

Effects of increasing hydrogen ion concentration on humoral intermediation. Though our observations on this point are mainly qualitative they definitely show that a diminishing pH of the environment, within limits not yet established,

increases the response of the rectus abdominis to extraneous acetylcholine. This holds for Ringer's solution acidified with either carbon dioxide or NaH_2PO_4 . The most striking effect that we have so far obtained is shown in figure 5 in which the pH of Ringer's solution was adjusted in four stages with NaH_2PO_4 , from 7.50 to 5.40 (pH determinations were made with a glass electrode). As a precaution to obtain equal concentrations of acetylcholine in the environment the acetylcholine was added to each solution immediately preceding each immersion of the muscle. The reciprocal of the height of contractions illustrated in figure 5 plotted against the prevailing pH, yields a curve roughly comparable to that of hydrolysis of acetylcholine by cholinesterase against pH, as published by Glick (1937) (see his fig. 1). That a common factor is involved in Glick's in vitro experiments and in our experiments on the living muscle seems at least suggested. Correspondence between the tissue and the in vitro experiments within a physiological range of pH indicates that the acid humoral mechanism of intermediation of stimulation may constitute a biologically significant adjustment of nervous integration.

Intra and extra cellular hydrogen ion concentration. Artificial environments of relatively high pH values are capable of transferring acid effects across a cellular membrane to the interior of a living cell, provided the environment possesses a high acid content (CO_2 in the case of Jacob's experiments). These observations of Jacobs (1920) showing that intra and extra cellular cH are not necessarily equal, even though equilibrium across the membrane be established, has been of great service in clarifying the mechanisms of the chemical control of breathing. Combined with the acid humoral concept of intermediation of stimulation it now promises new concepts of nervous integration.

It is recalled that preacidulation of the excised heart of the turtle with carbonated Ringer's solution intensifies the inhibitory action of exogenous acetylcholine (Gesell, Mason and Brassfield, 1944). In the present experiments preacidulation of the rectus abdominis is also found to intensify the response to extraneous acetylcholine just as simultaneous acidulation does, i.e., immersion of the muscle in a carbonated acetylcholine containing solution. The directional change of cH of the muscle or the direction of the movement of carbon dioxide seems to play no rôle in the response of the muscle, for in the preacidulation experiments the muscle is actually turning more alkaline as acetylcholine enters the muscle, whereas in the simultaneous acidulation experiments the muscle is turning more acid as the acetylcholine enters its substance. In both instances, however, the muscle is more acid than normal when acetylcholine enters. Since in one instance the muscle is more acid than the acetylcholine containing environment and in the other more alkaline, the results are taken to indicate that intra rather than extra cellular cH is the determining factor.

Additional data on this point are presented in contractions 6A, 6B, 6C and 6D, occurring during brief and equal immersions of the rectus in specially prepared buffer solutions to which equal quantities of acetylcholine were added. These solutions will be referred to as 6A, 6B, 6C and 6D. Ringer's solution serves as the base of all. Solution 6A is an unmodified Ringer's solution with a pH of

7.38. Solution 6B is Ringer's solution plus 0.15 per cent NaHCO_3 saturated with carbon dioxide to a pH of 5.72. Solution 6C is prepared by the addition of NaHCO_3 to solution 6B and has a pH of 6.50. Solution 6D is prepared by the addition of Na_2CO_3 to solution 6B and has a pH of 6.30. After each contraction the muscle is flooded with plain Ringer's solution.

It will be seen that there is no simple relation between the pH of the environment of the muscle and the height of the contractions recorded. Contractions 6B and 6C occurring at a pH of 5.72 and 6.50 are higher than that of 6A occurring at a greater pH of 7.38. Also contractions 6C and 6D at pH 6.50 and pH 6.30 are lower than contraction 6B at pH 5.72. Contraction 6C, however, is higher than that of 6D despite the fact that the environment is 0.2 pH less acid than that of 6D.

This latter finding would seem to emphasize the importance of the composition of the buffered environment as contrasted with its resultant pH. The excess of NaHCO_3 in solutions 6C and 6D as compared with solution 6B might be expected to promote a freer diffusion of base into the muscle and turn it more alkaline and thus tend to lower the response to acetylcholine. The presence of free CO_2 should on the contrary exercise the opposite effect of turning the muscle acid. Since the pressure of carbon dioxide must, however, be higher in solution 6C than in solution 6D it might be expected to promote a freer diffusion of CO_2 into the muscle and thus more effectively counteract the predominant alkaline effects of excess NaHCO_3 . If this interpretation is correct these findings re-emphasize the importance of intracellular acidity as contrasted with extracellular acidity.

The effects of intravenous injection of NaHCO_3 and Na_2CO_3 noted by Gesell and Hertzman (1926) in the dog assume a new interest in this connection. While both salts were observed to lower the cH of the blood they produced opposite effects upon the cH of the cerebrospinal fluid. Bicarbonate which increased the cH of the cerebrospinal fluid increased the volume of breathing whereas carbonate which lowered the cH inhibited breathing. It therefore seems probable that the opposite effects of these salts on breathing are also interpretable in terms of intracellular cH as indicated by the cerebrospinal fluid. The relatively small amount of cholinesterase in the blood as compared with the large amounts in motor end plates and synapses suggests why acid should function most effectively as an anticholinesterase intracellularly.

DISCUSSION. Similarities of response of *Nitella* to localized chemical irritation and of striated muscle fibers to exogenous acetylcholine suggest analogies of neurophysiological interest (see fig. 2). According to Osterhout and Hill (1930) the rhythmic activity of *Nitella* is created by electrotonic currents which stimulate the membrane at their site of outflow. The periodic activity which originates immediately outside the area of irritation is longitudinally propagated in two directions in the manner proposed by Lillie for conduction in nerve and muscle fibers. This rhythmic discharge resembles and, so far as is known to us, is equivalent to the well known rhythmic response of the isolated ventricle to a galvanic current, the "closing tetanus" of nerve and the rhythmic discharge of

nerve fibers to their own currents of injury. According to Buchthal and Linhard the rhythmic contractions of striated muscle produced by exogenous acetylcholine, like the rhythmical electrical response of *Nitella* to chloroform, originate in a highly localized source of stimulation, namely, the motor end plate. In our opinion the tetanic contractions of a muscle fiber produced by exogenous acetylcholine represent a response to an electrotonic current created and maintained by a steady supply of exogenous humor at the motor end plate (see fig. 2 and Gesell, Brassfield and Hansen, 1942).

Under physiological conditions muscle fibers do not function as rhythmical end organs due to the rapid destruction of acetylcholine liberated by a single nerve impulse. Preservation of the life span of such a liberation of endogenous acetylcholine by eserization however changes the single response to a single nerve impulse into a repetitive contraction. This repetitive response, in our opinion, is created by the prolongation of the electrotonic current set up at the motor end plate and, as far as we are able to judge, is similar in essentials to the after-discharge of nerve cells which have been bombarded by nerve impulses (Gesell, Brassfield and Hansen, 1942). The recent observations of Kuffler (1942) showing that eserization of muscle actually prolongs motor end plate potentials would seem to fall in line with the electrotonic concept of stimulation of muscle.

It was observed above that the contraction of a muscle subjected to a steady source of exogenous acetylcholine continues for a considerable time after the muscle is transferred to an acetylcholine-free environment. The relatively slow destruction and outward diffusion of the acetylcholine harbored by the muscle would explain this after activity. If this be true, the progressive weakening of contraction in acetylcholine free Ringer's solution presents the simplest conceivable example of the phenomenon of "after discharge" such as is observed in nerve cells. On that basis the declining "after discharge" of reflex centers becomes a function of the destruction and outward diffusion of the endogenous acetylcholine liberated by a preceding sensory stimulation, a concept which is in agreement with the humoral nature of after discharge as postulated by Rosenblueth (1935).

Support of these views is found in the observations that chemical factors which are supposed to delay the destruction of acetylcholine prolong "after discharge" in several cholinergic systems including the respiratory center, the heart and the respiratory muscles. Eserine and acid, for example, intensify and prolong the inhibitory effects of exogenous and endogenous acetylcholine on the heart (Gesell, Mason and Brassfield, 1944). Eserine brings on a repetitive response of striated muscle to a single motor impulse and acid prolongs the after response to exogenous acetylcholine. Both eserine and hypercapnia prolong "after hyperpnea" (i.e., reflex after discharge of the respiratory center) produced by faradic stimulation of sensory nerves (Hering's nerve and sensory cutaneous nerves) (Gesell, Hansen and Worzniak, 1943; Gesell, Hansen and Brassfield, 1942). The highly interesting observations of Eccles (1943) showing that increased frequency of stimulation of primary neurones intensifies, smoothes and prolongs

the synaptic potentials of secondary nerve cells would seem to support our concepts of the electrotonic mode of muscle stimulation and the electrotonic nature of nerve cell stimulation and nerve cell after-discharge. This intensification of synaptic potentials (our electrotonic current, Gesell, 1940) we believe is due to a "pooling" of intrinsic acetylcholine similar to that which occurs during hypercapnia (Gesell, Brassfield and Hamilton, 1942) and eseriniziation (Gesell, Brassfield and Hansen, 1942; Gesell and Hansen, 1943). Hypercapnia and eseriniziation which are thought to prolong the life cycle of an individual synaptic liberation of acetylcholine should have much the same effect as that of increasing the frequency of stimulation.

SUMMARY AND CONCLUSIONS

The contraction of the rectus abdominis muscle submerged in an acetylcholine containing environment consists of two phases of shortening—a brief and rapid shortening followed by a protracted and gradual shortening.

Replacement of the muscle in an acetylcholine free environment shows a moderately protracted period of diminishing contraction.

The rapid primary contraction produced by acetylcholine solutions is thought to represent an immediate response of those muscle fibers possessing superficially exposed motor end plates. The secondary protracted contraction is believed to express a progressive recruitment of new muscle units due to a gradual penetration of acetylcholine into the muscle.

Carbonation of the acetylcholine containing environment caused an increased height of contraction. The "after-contraction" occurring on transferring the muscle to a carbon dioxide free and acetylcholine free environment was also prolonged.

It is suggested that these augmenting and prolonging effects of carbon dioxide on the response of muscle to exogenous acetylcholine are importantly related to the anti-cholinesterase activity of the acid. Protection of the exogenous acetylcholine diffusing through and harbored within the muscle permits a deeper penetration of the humor and a consequently greater recruitment of muscle units. This same protection prolongs the "after-contraction" as well.

The phenomenon of "after-contraction" as observed in these experiments on exogenous acetylcholine is likened to the repetitive response of an eseritized muscle to endogenous acetylcholine liberated by a single motor nerve impulse. It is regarded as analogous to the "after-discharge" of reflex centers to sensory stimulation. These various forms of after activity are all believed to represent a periodic response to an electrotonic current generated by a common neuro-effector.

REFERENCES

- BRASSFIELD, C. R. AND R. GESELL. *Fed. Proc.* 1: 10, 1942.
BROWN, G. L. AND A. M. HARVEY. *J. Physiol.* 99: 379, 1941.
BUCHTHAL, F. AND J. LINHARD. *J. Physiol.* 90: 82P, 1937.
DALE, H. H., W. FELDBERG AND M. VOGT. *J. Physiol.* 86: 353, 1936.
ECCLES, J. C. *J. Physiol.* 101: 465, 1943.

- ECCLES, J. C., P. KATZ AND S. W. KIFFLER. *J. Neurophysiol.* **5**: 21, 1942.
- GESELL, R. *Ergebn. d. Physiol.* **43**: 477, 1940.
- GESELL, R., C. R. BRASSFIELD AND M. A. HAMILTON. *This Journal* **136**: 604, 1942.
- GESELL, R., C. R. BRASSFIELD AND E. T. HANSEN. *Fed. Proc.* **1**: 29, 1942.
- GESELL, R. AND E. T. HANSEN. *This Journal* **139**: 371, 1943.
- GESELL, R., E. T. HANSEN AND J. J. WÖRZNIAK. *This Journal* **138**: 776, 1943.
- GESELL, R. AND A. B. HERTZMAN. *This Journal* **78**: 610, 1926.
- GESELL, R., A. MASON AND C. R. BRASSFIELD. *This Journal* **141**: 312, 1944.
- GLICK, D. *Biochem. J.* **31**: 521, 1937.
- JACOBS, M. H. *This Journal* **53**: 457, 1920.
- KUFFLER, S. W. *J. Neurophysiol.* **4**: 209, 1941.
- OSTERHOUT, W. J. V. AND L. E. HILL. *J. Gen. Physiol.* **13**: 459, 1930.
- ROSENBLUETH, A. AND H. G. SCHWARTZ. *This Journal* **112**: 422, 1935. *

ENERGY EXPENDITURE IN SWIMMING¹

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Although a knowledge of the energy expenditure in swimming is essential in assigning this activity a proper place in exercise programs, records of such investigations are few. It is also not uncommon to find statements regarding energy costs of swimming without reference to the type of stroke, the velocity attained or the proficiency of the swimmer; yet these factors are of prime importance and, if disregarded, statements regarding the cost of swimming become meaningless. The purpose of this study was to determine the amount of energy spent in various swimming strokes by men and women for speeds from 2 to 5.5 feet per second.

In 1919 Liljestrand and Stenström tested the back, breast, trudgeon and side strokes, but at low velocity and without consideration of the oxygen debt. Greene (1930), Schmelkes (1935-6) and Egolinskii (1940) studied the breast and crawl strokes. In 1940 Karpovich and LeMaistre subjected the breast stroke to analysis.

PROCEDURE. Twenty-four subjects were tested: 15 college men, 22 to 26 years of age, and 9 girls, ranging in age from 15 to 22 years. Of this number four men were selected as representative of good swimmers and tested repeatedly in order to permit the construction of individual speed-energy curves. Five types of swimming styles were investigated: crawl, back (or inverted crawl), side, breast and butterfly.

The swimming distance was from 60 to 120 feet; that is, once or twice the length of the indoor pool in which the tests were conducted. Actual swimming started with a push-off, the force of which was roughly proportional to the speed of swimming; a weak push-off was used for low velocities and a strong one for high speeds. Long glides were eliminated and subjects started the use of the arms and legs as soon as possible after the push-off.

During the swim the subject held his breath and, immediately upon completion of the exercise, the collection of expired air began. This continued for from 20 to 40 minutes, depending upon the intensity of the exertion. The Douglas-Haldane method of measuring metabolism was employed, and the amount of energy used was calculated from the oxygen debt. The subjects were tested in the morning, in the "basal" state. Both the pre-swimming and the recovery metabolism were tested with the subject in a comfortable sitting position and well covered.

RESULTS. The results of this investigation are graphically presented in four figures, showing the relation between the speed of swimming and the energy used. In each figure the curves represent energy expenditure for the selected good

¹ This study was aided by a grant from the Elizabeth Thompson Science Fund.

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swimmers who were tested over the whole speed range. As may be observed, the results obtained from other swimmers deviate from these curves, depending upon the proficiency of the individual.

For example, it may be seen in figure 1 that at a speed of 2.5 ft./sec. one subject used 70 cal./min., or her energy expenditure was almost 5 times as great as that indicated by the curve. At a speed of 5.5 ft./sec. the curve shows 96 cal./min., whereas a champion swimmer expended only 75 cal./min. Looking across the graph, it may be observed that this is just about the same amount expended by the poor swimmer at only 2.5 ft./sec.

Similarly in figure 2 an inefficient backstroke consumed 100 cal./min. at a speed of 3.8 ft./sec.; yet an expert used only 36 cal./min. These deviations, naturally, are expected in testing different people for any stroke and speed. However, in any stroke these deviations are smaller at the greater speeds than at the lesser speeds. The explanation is obvious: in order to develop high speed, swimmers must be in a state of good mechanical and physiological efficiency, which precludes any large fluctuations.

Analysis of the curve in figure 1 shows that the energy expenditure in the crawl stroke is roughly proportional to the square of the speed. This agrees with the previous investigation (Karpovich, 1933) in which the water resistance was found to vary approximately as the square of the speed. Although water resistance in the glide on the back also varies as the square of the speed, examination of the curve on figure 2 shows that in actual back stroke swimming the expenditure of energy rises faster than the square of the speed. This discrepancy may be due to additional water resistance caused by a certain body twisting typical for the back stroke.

In the breast and side strokes the relationship between the energy used and the speed of swimming is not as simple as in the crawl or even in the back stroke (figs. 3 and 4). The reason for this is a more complicated arm and leg action. During the recovery phases of the stroke, just before the arm thrust and leg kick, both legs and arms are in a very awkward position as far as streamlines are concerned, causing an excessive rise in water resistance. Although this is somewhat offset by a more efficient leg kick in breast and side strokes than in crawl and back, yet the total effect is a loss in efficiency and therefore a greater expenditure of energy.

With elimination of the underwater arm recovery in the butterfly, one cause of loss of energy has been also eliminated and strangely enough the relationship between the speed and the energy use is about the same as in the crawl.

For the sake of comparison, the data for back and breast strokes obtained by Liljestrand and Stenström (1919) were also added to the corresponding curves in figures 2 and 3. As can be seen, their data for speeds less than 2 ft./sec. seem to lie on the continuation of these curves; for greater speeds (see fig. 3) their figures are too low because Liljestrand and Stenström did not take into consideration the oxygen debt.

The relationship between breast and butterfly strokes is especially interesting. In the butterfly stroke, the arms are carried through the air in continuous rotary

motion, resembling a waterwheel. In this manner the resistance caused by the arms in recovery is eliminated, and greater speed is obtained. Yet it will be noted (fig. 3) that for speeds under 3 ft./sec. it costs more to swim butterfly style than breast, and a possible explanation for this may be the extra effort needed to preserve the body equilibrium which is disturbed at low speed by the simultaneous lifting of both arms.

It would be futile, however, to try to convince a swimmer that at greater speeds the butterfly stroke is more economical than the breast, because he knows from experience that the butterfly is more tiring. This paradox may be solved by con-

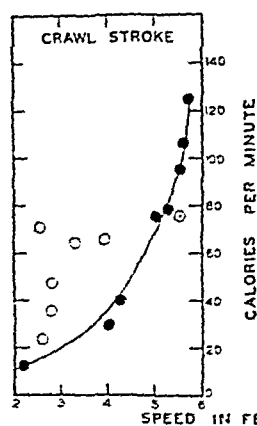


Fig. 1

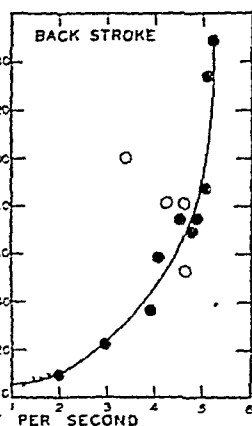


Fig. 2

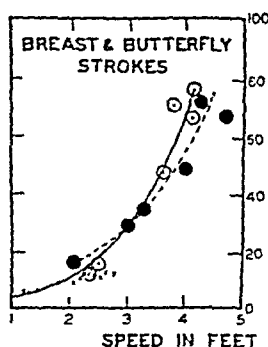


Fig. 3

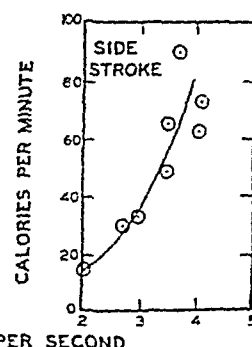


Fig. 4

Fig. 1. Energy expenditure in the crawl stroke. ●—Data, obtained on one good swimmer, from which the curve was plotted. ○—Other swimmers.

Fig. 2. Energy expenditure in the back stroke. ●—Data, obtained on one good swimmer, from which the curve was plotted. ○—Other swimmers. ×—Data taken from Liljestrand and Stenström (1919).

Fig. 3. Energy expenditure and the speed of the breast and butterfly strokes. ○—Breast stroke. ●—Butterfly stroke; one good swimmer was used for each stroke. ×—Data taken from Liljestrand and Stenström (1919). (Data obtained on 4 breast strokes and 4 butterfly swimmers are not shown here to avoid confusion. They all were mediocre swimmers and therefore the energy expenditure was a great deal higher than that indicated by the respective curves.)

Fig. 4. Energy expenditure and the speed of the side stroke. One good swimmer was tested.

sidering that the fatiguing effect in the butterfly stroke is mostly of local origin, being confined largely to the muscles of the shoulder girdle, which are in constant action. In the breast stroke, however, there are two periods of comparative rest for these muscles, one during the glide, and the other during the recovery phase. The relative energy cost of the various strokes thus depends on the rate of speed. At speeds greater than 3 ft./sec. it is possible to arrange swimming styles in order of increasing energy cost as follows: crawl, back, butterfly, breast and side. Under 3 ft./sec. the butterfly is less economical than the side.

Egolinskii (1940) found that for the same rate of speed the crawl stroke is more

economical than the breast stroke. Scrutiny of his data reveals that he does not have enough evidence to substantiate his deduction. He tested each of his subjects three times and obtained inconsistent results. His data were recalculated so that the relation between the speed of swimming and calories per minute could be established. Subject M. Sh., using the breast stroke, spent 48.5 cal./min. at a speed of 3.41 ft./sec. and only 37.9 cal./min. at a speed of 3.77 ft./sec. Subject L. M., using the crawl stroke, spent 51.1 cal./min. at a speed of 3.41 ft./sec. and only 39.6 cal./min. at a speed of 3.86 ft./sec. Since the expenditure of energy should rise with the increase of speed, these figures are inconsistent.

Schmelkes states that up to a speed of 3 ft./sec. the crawl requires more energy than the breast stroke. As there is no assurance that his swimmers were equally proficient in both styles, his deductions may be questioned.

As far as the comparative economy of the strokes is concerned, a rather simple additional argument may be offered. Greatest swimming speeds are obtained by champions with the crawl stroke, therefore the crawl stroke must be the most economical. At very low rates of speed this relation may be different, but, since in the present investigation the lower limit was a speed of 2 ft./sec., nothing can be said about the relative economy of the strokes below this speed.

Although the limit of power and speed was observed to be higher in boys than in girls, no sex difference was evident in the energy expenditure for the same strokes and speeds.

SUMMARY

1. The energy cost of swimming crawl, back, breast, butterfly and side strokes at speeds greater than 2 ft./sec. was found on twenty-four subjects of both sexes.

2. Swimming at speeds higher than 2 ft./sec. should be considered a vigorous exercise because metabolism rises to more than 10 times the basal rate. At speeds beyond 5 ft./sec. metabolism may be more than 100 times higher than basal. Unskilled swimmers expend from 2 to 5 times as much energy as skilled swimmers. Fluctuation between individuals is greater for the lower speeds than for the higher ones.

3. The swimming strokes may be arranged in order of increasing energy cost as follows: crawl, back, breast and side. This relationship holds true for any corresponding speed. The butterfly stroke, however, has certain peculiarities. It is the least economical of the five strokes under 2.5 ft./sec. Above this speed it becomes more efficient than the side stroke and at 3 ft./sec. it is more economical than the breast stroke.

4. A greater fatiguing effect of the butterfly stroke as compared with the breast style may be due to local fatigue of the shoulder girdle muscles.

5. No sex difference in the relative cost of swimming was observed.

We wish to thank Mr. Richard Pohndorf for his invaluable assistance in this research, and Mrs. David Heinlein for technical aid during the additional checking tests.

REFERENCES

EGOLINSKII, E. Jour. Physiol. U.S.S.R. 28: 700-6, 1940.

GREENE, M. The energy cost of track running and swimming. Master's Thesis, Springfield College, 1930.

KARPOVICH, P. Res. Quart. Am. Assn. Hlth. Phys. Educ. 4: 21, 1933.

KARPOVICH, P. AND H. LEMAISTRE. Res. Quart. Am. Assn. Hlth. Phys. Educ. 9: 40, 1940.

KARPOVICH, P. AND N. MILLMAN. Fed. Proceed. 1: 44, 1942.

LILJESTRAND, G. AND N. STENSTRÖM. Skand. Arch. f. Physiol. 39: 1, 1919.

SCHMELKES, B. Przegl. Fizjol. Ruchu 7: 201, 1935-36.

RADIOACTIVE PHOSPHORUS STUDIES ON HEXOSEMONO-PHOSPHATE METABOLISM IN RESTING MUSCLE¹

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The hexosemonophosphate (HMP) of striated muscle, first isolated by Embden and Zimmerman (3), was shown by Lohmann (5) to consist of an equilibrium mixture of glucose-6-phosphate (GP) and fructose-6-phosphate (FP), in which the GP predominates. Lohmann found that muscle extract contains an enzyme which converts either pure substance into the equilibrium mixture with extreme rapidity. He also showed (4) that the fructose derivative has a higher initial rate of hydrolysis by boiling mineral acid than does the glucose derivative. The two compounds are so similar in their physical properties that a separation by ordinary means is not feasible.

The use of radioactive phosphorus (P^{32}) as a tracer, together with this difference in the initial hydrolysis rates, makes it possible to study the separate metabolic functions of the two compounds. In the experiments presented here, this method has been applied to the study of the metabolism of these phosphorylated sugars in resting muscle of cats and frogs.

In experiments with pure GP and FP it was found that, at the optimum acidity for the precipitation of P as ammonium phosphomolybdate, approximately 2.5 N nitric acid, and the temperature of the boiling water bath, some 35 to 40 per cent of FP underwent hydrolysis in 1 to 1½ hours, as compared to 20 per cent of GP. The complete hydrolysis of either compound requires an extremely long heating, as Lohmann found. Thus by hydrolysis for this short period, separation of the precipitated phosphomolybdate, and prolonged hydrolysis of the material remaining in the filtrate, it is possible to obtain two fractions: in the first, the P originally present as FP is in larger amount; in the second, P derived from GP predominates. Although the difference in per cent hydrolysis in 1 or 1½ hours is not very great, in the experiments with P^{32} the differences in relative radioactivity between the two fractions which were found are far beyond possible experimental error.

The general procedure of the experiments was similar to that of the previous studies (6): the P^{32} in the form of Na_2HPO_4 was injected subcutaneously in the cats or into the ventral lymph sac in the frogs, and the muscles frozen 2, 4 or 24 hours later. The frogs were decerebrated a day prior to use; the cats were anesthetized with pentobarbital for the freezing of the muscles. The entire musculature of both hind legs was used in the frogs, and the gastrocnemius muscle in the cats. Trichloroacetic acid filtrates were prepared, using three

¹ Supported by a grant from the John and Mary R. Markle Foundation

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volumes of 15 per cent solution, and the various organic P compounds present separated by barium and differential acid hydrolysis.

The barium-insoluble fraction was worked up by the methods previously described (6) for the separation of the P present in the two acid-labile phosphate groups of adenosine triphosphate (ATP). The barium-soluble fraction, containing the HMP, was acidified to about 2.5 N with nitric acid, excess ammonium molybdate added, and the solution let stand overnight, to precipitate the trace of inorganic P present and that liberated by the hydrolysis of phosphocreatine. This precipitate was discarded, and the filtrate heated on the boiling water bath for 1 to 1½ hours, cooled and filtered. The filtrate was then heated on the steam bath for 40 hours to complete the hydrolysis of the HMP. All phosphomolybdate precipitates whose radioactivity was to be measured were converted to $\text{Mg-NH}_4\text{PO}_4$, and measurements of relative radioactivity made on the material thus obtained by the procedure previously described (6). The amounts of readily hydrolyzable P of ATP found corresponded to about 30 to 35 mgm. per cent, and the total HMP to about 7 or 8 mgm. per cent as P. These values are slightly lower than those obtained by direct colorimetric determination on trichloroacetic acid filtrates. The distribution of the P between the two fractions of the HMP varied considerably. The amounts of P from ATP which were obtained ranged from 1 to 2 mgm.; the P in each of the HMP fractions was usually between 100 and 200 gamma. Control experiments with 200 gamma of inorganic P, carried through the phosphomolybdate and magnesia mixture precipitations, gave recoveries of over 90 per cent. In the tables, the data on radioactivity have been expressed as counts per minute per milligram P, calculated to the basis of one million counts per minute per kilogram body weight, as of the day the determinations were made. Thus the figures on any two animals are directly comparable.

The results obtained lead to the following conclusions: the mechanism by which glucose enters the muscle cell is the formation of GP on the membrane, with penetration of the glucose portion into the cell interior accompanied by the hydrolysis of the phosphate linkage, the phosphate remaining in the extracellular phase; in the muscles of cats, but not in frogs, there is an interchange of phosphate groups in resting metabolism between ATP and FP; in the muscles of frogs, but not in cats, FP and GP are interconvertible; in the muscles of neither species does transfer of phosphate groups take place between ATP and GP; the administration of appreciable amounts of inorganic phosphate to fasted, but not to post-absorptive, cats, leads to a mobilization of GP on the cell membrane; and the administration of glucose with the phosphate in fasted cats increases this mobilization of GP on the muscle cell membrane.

In earlier experiments on the time course of the distribution of P^{32} in resting metabolism (6), an accumulation of HMP was found 2 hours after giving the tracer, when the total amount of carrier P amounted to several milligrams per kilogram. In the present experiments it was found, both in fasted cats (table 1) and in frogs (table 2), that this accumulation consists entirely of GP. Such a result might have been anticipated, since no fructose circulates in the body

fluids. However, the finding demonstrates that interconversion of the two compounds does not take place on the cell membrane. In the experiments on fasted

TABLE 1

Time course of uptake of P^{32} by acid labile groups of adenosine triphosphate, fructose-6-phosphate, and glucose-6-phosphate in resting muscles of fasted cats

Values are expressed in terms of counts per minute per milligram P, calculated to the basis of 10^6 counts injected per minute per kilogram body weight, as of the day of measurement.

ADENOSINE TRIPHOSPHATE	FRUCTOSE-6- PHOSPHATE	GLUCOSE-6- PHOSPHATE
2 hours after P^{32}		
38	65	124
80	84	202
66	27	149
79	37	189
88	102	209
Av... 70	63	175
4 hours after P^{32}		
75	31	114
84	14	167
52	19	170
77	25	219
78	60	228
65	56	250
70	45	217
Av... 72	36	195
24 hours after P^{32}		
193	132	84
167	122	102
165	121	88
224	150	121
Av....187	131	99

TABLE 2

Uptake of P^{32} by resting muscles of frogs. Values are in the same terms as in table 1

ADENOSINE TRIPHOSPHATE	FRUCTOSE-6- PHOSPHATE	GLUCOSE-6- PHOSPHATE
2 hours after P^{32}		
73	82	348
23	62	152
84	154	435
136	144	472
Av... .79	111	352
24 hours after P^{32}		
233	142	134
306	155	141
152	100	99
106	62	65
Av... 199	115	109

cats this accumulation of GP is still present four hours after the administration of P^{32} . However, by 24 hours after the injection, the P^{32} content of the GP has decreased to a value lower than that at 2 or 4 hours, while the P^{32} content of the ATP and FP have risen to values which now exceed those for the GP. If the

high P^{32} content of the GP at 2 and 4 hours did consist of intracellular material, the relative radioactivity could not drop to a lower value at a later time. Hence the high P^{32} content of GP must signify material which has not penetrated into the cell interior, and most likely is adsorbed on the membrane. Also, since the P^{32} contents of FP and ATP are higher than those of GP at 24 hours, there cannot have been any interchange of phosphate groups between GP on the one hand and FP or ATP on the other. This applies to indirect interchange as well as to direct.

In the 24-hour experiments on fasted cats, the P^{32} content of the FP is higher than that of GP, although lower than that of ATP. Since the hydrolysis procedure does not give a clean cut separation of the P of the two sugar derivatives, the actual P^{32} content of the FP must be higher, and that of the GP lower, than the measured values. The conclusion is therefore warranted that there has been interchange of phosphate groups between ATP and FP, and that they are in equilibrium. It is impossible to determine whether the interchange is direct or indirect, through inorganic phosphate or some other intermediate.

The situation in the 24-hour frogs is quite different. Here the P^{32} contents of FP and GP are equal, at a value significantly lower than that of ATP. Therefore FP and GP have been interconverted, presumably by the enzyme described by Lohmann (5). However, neither has exchanged phosphate groups, directly or indirectly, with ATP. The data on the two species point to two separate and distinct pathways of phosphorylating carbohydrate metabolism in resting muscle. Thus a situation which was already very complicated has become even more complex. It would be interesting to determine which of these two relations is found in other mammalian species.

The experiments described above were carried out on cats which had been last fed late in the afternoon of the second day previous to the morning on which the P^{32} was injected. Another series of experiments was then carried out on cats which were last fed late in the afternoon preceding the day of injection of the P^{32} , except that the 24-hour animals were fed again some 8 hours after the P^{32} . These animals will be considered as being in the post-absorptive state at the time of the experiment. As shown in table 3, there is a marked contrast between these animals and the fasted ones. There is practically no evidence for any mobilization of GP on the membrane in the post-absorptive animals at any of the times studied. The ATP in the 2 and 4-hour experiments, and all three compounds in the 24-hour ones, have a higher P^{32} content than in the fasting animals. These data also indicate exchange of phosphate groups between ATP and FP, but not between either of these and GP. The higher uptake of P^{32} must indicate a greater metabolic turnover of all these compounds in the post-absorptive state than in fasting. For the GP and ATP, this must mean greater rate of formation and synthesis of the compounds on the cell membrane. The absence of any mobilization of GP on the membrane signifies that phosphate administration is able to evoke such a mobilization only in the previously fasted animal.

This last point is emphasized even more strongly when the effect of glucose administration on the uptake of P^{32} is compared in the fasted and post-absorp-

tive animal (table 4). In these experiments, 30 cc. per kgm. of 5 per cent glucose solution was injected intraperitoneally 30 minutes after the injection of the P^{32} , so that the peak of blood sugar rise would come at the same time as the peak of P^{32} in the plasma inorganic phosphate. The muscles were frozen 4 hours after the

TABLE 3

Uptake of P^{32} by resting muscles of cats in post-absorptive state

Values in same units as previous tables

ADENOSINE TRIPHOSPHATE	FRUCTOSE-6- PHOSPHATE	GLUCOSE-6- PHOSPHATE
2 hours after P^{32}		
108	59	42
99	57	53
105	58	37
145	73	58
140	64	45
Av....119	62	43
4 hours after P^{32}		
172	110	67
115	75	55
150	133	73
143		47
98	61	77
132	76	59
Av....135	91	63
24 hours after P^{32}		
229	210	111
225	216	142
249	197	118
195	194	142
Av....225	204	128

TABLE 4

Effect of glucose administration on P^{32} uptake by resting muscles of cats in fasting and post-absorptive states

Samples obtained 4 hours after injection of P^{32} , $3\frac{1}{2}$ hours after glucose. Values in same units as in previous tables

ADENOSINE TRIPHOSPHATE	FRUCTOSE-6- PHOSPHATE	GLUCOSE-6- PHOSPHATE
Fasted		
106		665
84	50	610
72	103	385
83	72	590
63		402
73	38	525
Av....80	66	530
Post-absorptive		
58	55	38
76	51	37
87	67	70
92	50	94
56	17	30
51	32	47
87	68	60
Av....74	49	54

injection of the P^{32} . In the fasted animals, the glucose has evoked about three times as great an accumulation of GP on the membrane as the phosphate alone; in the post-absorptive animals, the glucose has not brought about any change in the P^{32} content of the GP different from that resulting from phosphate alone. It has, however, reduced the uptake by the ATP. The significance of this latter point will be discussed in a later paper.

These data are of value in relation to the mechanism of glucose absorption by the muscle cell, and to the conditions under which such absorption takes place. The free glucose of striated muscle in the rat is about one-eighth that of plasma, and that of the heart about one-third the plasma content (2). These fractions correspond very closely to the fractions of these two organs which have been found (1,7) to comprise the extracellular phase. The inference is therefore permissible that all the free sugar in these tissues is present in the extracellular phase, and that the cell membrane is impermeable to glucose. The absorption of glucose into the muscle cell must therefore take place through the formation on the membrane of some compound of which the glucose portion at least is able to penetrate into the cell interior. The experimental data here presented indicate very strongly that GP is the compound involved, and further indicate that absorption of glucose by muscle is a function of the carbohydrate reserves as well as of the glucose supply. The accumulation of GP on the membrane in the fasting animal when phosphate is given, the marked increase in this accumulation produced by glucose, and the failure of this process to take place in the post-absorptive animal, all point strongly to the carbohydrate reserves playing an important part in determining the extent of glucose absorption.

The presence of P^{32} in the GP under all conditions shows that there must be some metabolic turnover of this compound, with formation on the membrane, penetration of the molecule into the cell interior, and the reverse processes taking place constantly. However it seems clear that this metabolic turnover must be distinguished sharply from the accumulation of GP on the membrane that is involved in glucose absorption. The data show that the phosphate groups which are active in glucose absorption do not necessarily penetrate the membrane, but remain extracellular. The previously published data (6) which showed that no interchange of phosphate groups takes place, directly or indirectly, between intracellular HMP and intracellular inorganic phosphate, together with the present findings that in the cat this failure of interchange is limited to the GP fraction of the HMP, indicate that the penetration of the glucose portion of the GP molecule into the cell interior is accompanied by hydrolysis of most of the phosphate, and that the resulting inorganic phosphate remains extracellular. On this basis, the P^{32} content of the GP in tracer experiments cannot be used as a measure of the amount of glucose absorption taking place, but only as an indication as to whether it is taking place to any significant extent.

The writer wishes to express his thanks to Dr. J. G. Hamilton of the Radiation Laboratory, University of California, and to Prof. J. M. Cork, of the Department of Physics, University of Michigan, for their generous supplies of radioactive phosphorus.

SUMMARY AND CONCLUSIONS

1. Radioactive phosphorus has been used to study the metabolism of glucose- and fructose-6-phosphates in the resting metabolism of striated muscle of cats and frogs.

2. In the muscles of frogs, but not in those of cats, the two phosphorylated sugars are directly interconvertible.
3. In the muscles of cats, but not in those of frogs, there is ready interchange of phosphate groups, either direct or indirect, between fructose-6-phosphate and adenosine triphosphate.
4. Neither in cats nor in frogs is there any interchange of phosphate groups between glucose-6-phosphate and adenosine triphosphate.
5. In fasted cats, the administration of phosphate leads to a mobilization of glucose-6-phosphate on the muscle cell membrane.
6. The administration of glucose to fasted cats increases markedly the phosphate-induced mobilization of glucose-6-phosphate on the muscle cell membrane.
7. Phosphate does not lead to a mobilization of glucose-6-phosphate on the cell membrane of cats in the post-absorptive state, nor does glucose administration to such animals evoke such mobilization.
8. Evidence is presented that the absorption of glucose by the muscle cell takes place through the formation of glucose-6-phosphate on the cell membrane, penetration of the glucose portion of this molecule through the membrane, followed by hydrolysis of the phosphate linkage, with the phosphate remaining in the extracellular phase.
9. In resting muscle, absorption of glucose apparently takes place only when the carbohydrate reserves have been depleted, as by fasting.

REFERENCES

- (1) AMBERSON, W. R., T. P. NASH, A. G. MULDER AND D. BINNS. *This Journal*¹ **122**: 224, 1938.
- (2) CORI, G. T., J. O. CLOSS AND C. F. CORI. *J. Biol. Chem.* **103**: 13, 1933.
- (3) EMBDEN, G. AND M. ZIMMERMAN. *Hoppe-Seyler's Ztschr.* **167**: 114, 1927.
- (4) LOHMANN, K. *Biochem. Ztschr.* **194**: 306, 1927.
- (5) LOHMANN, K. *Ibid.* **262**: 137, 1933.
- (6) SACKS, J. AND C. H. ALTSHULER. *This Journal* **137**: 750, 1942.
- (7) YANNET, H. AND C. H. DARROW. *J. Biol. Chem.* **134**: 721, 1940.

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THE INFLUENCE OF GLYCOTROPIC SUBSTANCES ON SURVIVAL OF THE PRIMITIVE RESPIRATORY CENTER IN THE ISCHEMIC RAT HEAD¹

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Selle (1, 2, 3) has shown that the duration of survival of the decapitated rat head depends upon age, previous treatment with drugs, and temperature. He believes that following decapitation in young rats only, two series of gasps separated by a period of apnea occur. Our results do not corroborate this phenomenon in its entirety for, although the second series diminishes to a statistical zero at approximately 60 grams weight, there is in most animals a recurrence of these later gasps to a lesser degree from about 90 grams to 500 grams (oldest animal used). That the late series is anaerobic is evidenced partly from the fact that survival is roughly inversely proportional to age agreeing therefore with the anaerobic findings in the newborn (4, 5, 6), and partly from the use of substances preventing oxidative and non-oxidative reactions (2, 7, 8).

The purpose of this investigation was to determine the effects of glycotropic substances on survival time of the decapitated rat head as measured by gasping. Since the source of this energy, both central and peripheral, is undoubtedly glycolytic (6, 8, 9, 10) empirically then glycogenic substances (insulin) should cause decrease in survival time by the resulting hypoglycemia, and glycogenolytic substances (adrenaline, ephedrine) and substances causing glycogenesis (pituitary factors) should prolong survival time due to the resulting hyperglycemia. Since the degree of anition should also influence duration of survival, especially after the application of the glycotropic substances, both starved and unstarved rats were used. In all cases of starvation rats were deprived of food for a period of 22 hours but were supplied with water ad libitum. Male rats only of the Wistar strain were used and these of approximately 21 days of age (average weight of 45 grams), this age being chosen because of similar survival times of different control animals in this age group. Heads were amputated by single edge razor blades held vertically in a blade holder. Gasps were recorded manually on an electric kymograph turning at a uniform speed. Only gasps were recorded, muscular twitchings being ignored. Each drug was injected

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intraperitoneally and a certain time allowed for assimilation and pharmacological action. Drugs used, dosage, and lapse of time after injection were as follows:

Drug	Dosage (mgm.)	Time lapse (min.)
Adrenaline HCl.....	0.045	10
Insulin.....	1.2 (units)	30
Iodoacetic acid.....	2.0	15
Anterior pituitary.....	2.5	15
Posterior pituitary.....	2.5	15
Ephedrine HCl.....	5.0	10

In the non-premedicated animal there are usually two series of gasps but following adrenaline injection a third series differentiates (see table 1 and fig. 1) which

TABLE 1

Showing average total survival time, average duration and number of gasps, and per cent change from normal survival time

	NUMBER OF ANIMALS	TOTAL SURVIVAL TIME	EARLY SERIES		INTERMED. SERIES		LATE SERIES		PER CENT CHANGE FROM NORMAL
			Duration	Number of gasps	Duration	Number of gasps	Duration	Number of gasps	
		<i>seconds</i>	<i>seconds</i>		<i>seconds</i>		<i>seconds</i>		
Normals (N).....	24	28.8	5.6	5.3			4.0	1.7	
Normals (S).....	24	26.3	5.9	7.5			2.9	1.9	
Adrenaline (N).....	11	43.9	7.8	7.2	4.6	2.3	5.2	3.7	+53
Adrenaline (S).....	12	31.5	8.5	8.4	1.9	1.7	2.5	1.7	+20
Insulin (N).....	10	22.1	8.1	7.8			2.1	1.7	-23
Insulin (S).....	11	11.2	7.9	6.8					-57
Iodoacetic (N).....	10	17.8	3.8	4.0			2.3	1.5	-38
Iodoacetic (S).....	8	11.3	7.4	5.9					-57
Ant. pituitary (N).....	12	51.2	5.2	5.2			25.0	6.8	+77
Ant. pituitary (S).....	12	38.1	2.9	2.4			10.5	3.6	+45
Post. pituitary (N).....	18	44.0	0.9	3.6			18.5	5.8	+54
Post. pituitary (S).....	18	39.6	6.6	5.7			4.1	2.6	+37
Ephedrine (N).....	12	21.8	12.6	11.6			0.5	0.5	-24
Ephedrine (S).....	12	17.7	9.7	9.8			1.3	0.5	-33

(N) denotes non-starved animals; (S) denotes those starved 22 hours.

appears to be intermediate to the first and last series. With no other drug used in this investigation did we obtain three series of gasps although possibly other sympathomimetics produce the same effect. Since adrenaline causes hyperglycemia the extra series of gasps is likely due to glycolytic activity and may well be anaerobic. One might even assume that all gasps of the ischemic head are anaerobic since circulatory stasis occurs instantly and a few seconds elapse during the apneic pause before the first gasp appears.

The results are summarized in table 1, and shown graphically in figure 1, the latter showing 1, average survival time; 2, average duration of gasping, and 3, spatial distribution of gasping periods. It will be noted that adrenaline greatly

prolonged the total survival time especially in the non-starved animals, also causing the additional period of gasping. The physiology of this phenomenon appears mysterious but undoubtedly a different enzyme system is involved. Insulin markedly reduced the total survival time, especially in the starved animals, the great difference here being due to the total absence of the delayed period of gasps. If one assumes that the delayed gasps are anaerobic it is here quite evident that the anaerobic energy is from glycolysis. Iodoacetic acid produces effects similar to those of insulin in that the delayed series of gasps is absent in fasted rats. Being an enzyme inhibitor in the anaerobic cleavage of glucose iodoacetic acid further strengthens the evidence for the anaerobic nature of the delayed gasps.

Because it is generally acknowledged that pituitary extracts cause a transient rise in blood sugar, anterior and posterior pituitary gland aqueous extracts were injected, resulting in definite prolongation of survival time as well as an increase

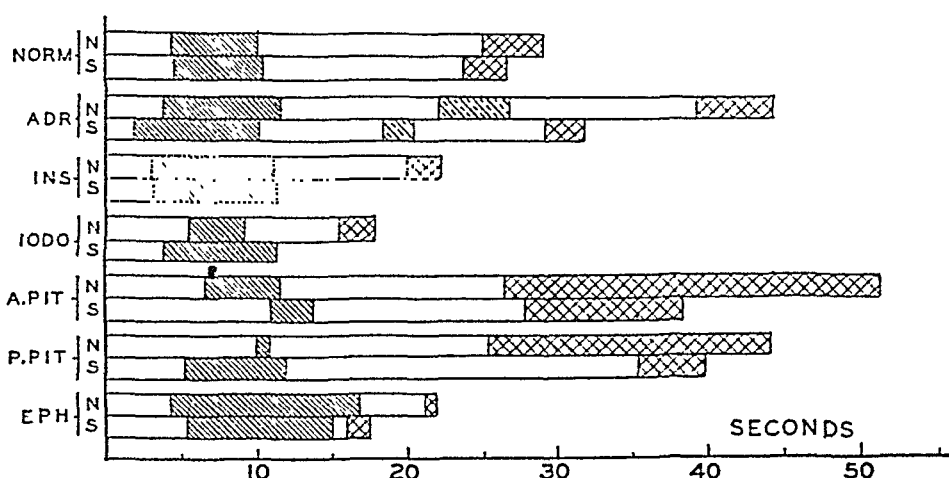


Fig. 1 Showing average total survival time, average duration and relative position of gasping periods. Shaded areas are periods of gasping, clear areas are periods of apnea. S, starved 22 hours; N, non-starved.

in the duration of gasping. The striking similarity in the late series of gasps shown by careful study of the kymographic tracings of the effects of the anterior and posterior lobe extracts strongly suggests a similar identity of the two substances involved. There are, however, two possibilities of explanation; either 1, the dried gland products as prepared by the pharmaceutical supply company contain impurities such that possibly the anterior lobe diabetogenic principle is contained in each product, or 2, that a separate glycotropic principle, per se, exists in the posterior lobe which is separate from the diabetogenic factor of the anterior lobe. In all of the pituitary treated animals the final period of gasping is marked by longer than normal periods between successive gasps thereby causing a marked increase in the total length of this period although the total number of gasps may not be correspondingly greater.

Ephedrine, known to potentiate the action of adrenaline by destruction of the amine oxidase system was suspected to have some effect on glycemia and sur-

vival time. As the data show it greatly increased the initial period of gasping (approximately 70 per cent) but shortened the total survival time (24 per cent). In other words the additional energy was quickly consumed and little was left for the delayed period. Because ephedrine also is a medullary excitant it might be argued that the increased gasping could at least be partially accounted for by a hyper-sensitive medullary respiratory center.

Conditions affecting survival of the decapitated head and those encountered in the anoxia of decompression (high altitudes) are not necessarily identical. We have found this true (unpublished data) in respect to adrenaline, exclusive carrot diet, water starvation, inanition, etc. It has been stated (11) that certain cholinergic and sympathicolytic agents increase, while adrenergic and parasympathicolytic agents decrease resistance to anoxia resulting from barometric decompression. Others (12) have reported that ephedrine and similar synthetic related compounds delay respiratory failure in animals exposed to sudden anoxia.

The occurrence of definite gasping periods separated by apneic pauses might well be explained by the existence of different levels of integration of the centers. The primitive center being the most rugged should persist the longest in anoxia and, therefore, the final series of gasps probably represents activity of this center alone. Evidence for this is shown by two facts: 1, the slow rhythmic discharge of the final series of gasps, and 2, their definite apneustic character. Thus probably the earlier series of gasps being of a more rapid nature and less apneustic represents the superimposed regulation of the higher centers. Because the higher centers are most susceptible to anoxic degradation they should be expected to fail first and the subsequent and final rhythm would be that of the lowest (apneustic) center. Section of the head from the body instantly severs any vagal connections with the respiratory center thereby eliminating any reflex influence from the lungs.

CONCLUSIONS

Glycotropic substances affecting survival time, duration and number of gasps of the decapitated rat head are adrenaline, insulin, iodoacetic acid, anterior and posterior pituitary extracts, and ephedrine. Starvation modifies the results of these substances as less glucose becomes available. An additional series of gasps appears after adrenaline injection. The delayed period of gasps is entirely abolished in starved animals treated with insulin or iodoacetic acid indicating the anaerobic nature of this series of gasps. Aqueous extracts of anterior or posterior pituitary gland greatly prolong the survival time showing effects qualitatively similar. Survival time is increased by adrenaline, anterior or posterior pituitary, but shortened by ephedrine, insulin, and iodoacetic acid.

Addendum. After this investigation was completed a paper appeared by Selle (13) in which it was shown that insulin and glucose have practically the reverse effects. Glucose given to animals under the influence of large doses of insulin restores the gasping pattern to nearly normal within 60 minutes. Selle's findings, therefore, are in agreement with ours regarding glycotropic influences.

REFERENCES

- (1) SELLE, W. A. Proc. Soc. Exper. Biol. and Med. 51: 50, 1942.
- (2) SELLE, W. A. Proc. Soc. Exper. Biol. and Med. 54: 291, 1943.
- (3) SELLE, W. A. Fed. Proc. 3: 40, 1944.
- (4) KABAT, H. This Journal 130: 588, 1940.
- (5) FAZEKAS, J. F., F. A. D. ALEXANDER AND H. E. HIMWICH. This Journal 134: 281, 1941.
- (6) HIMWICH, H. E., A. D. BERNSTEIN, H. HERRLICH, A. CHESLER AND J. F. FAZEKAS. This Journal 135: 387, 1942.
- (7) HIMWICH, H. E., J. F. FAZEKAS AND F. A. D. ALEXANDER. Proc. Soc. Exper. Biol. and Med. 46: 553, 1941.
- (8) HIMWICH, H. E., J. F. FAZEKAS AND E. HOMBURGER. Endocrinology 33: 96, 1943.
- (9) VAN MIDDLESWORTH, L., R. F. KLINE AND S. W. BRITTON. This Journal 140: 474, 1944.
- (10) FAZEKAS, J. F. AND H. E. HIMWICH. This Journal 139: 366, 1943.
- (11) EMERSON, G. A. AND E. J. VAN LIERE. J. Lab. Clin. Med. 28: 700, 1943.
- (12) BINET, L. AND M. STRUMZA. Compt. rend. Acad. Sci. 207: 543, 1938.
- (13) SELLE, W. A. This Journal 141: 297, 1944.

CIRCULATORY CHANGES FOLLOWING THE SUBCUTANEOUS INJECTION OF HISTAMINE IN DOGS^{1,2}

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The observations of Dale and his associates (Dale and Laidlaw, 1910) on the profoundly hypotensive effect of intravenously injected histamine in cats and dogs have been of especial interest to clinicians and physiologists because of the suggested rôle of histamine or related substances in the etiology of traumatic shock. Histamine poisoning has been studied extensively with relation to both the mechanism and therapy of shock (Dale, Laidlaw and Richards, 1919; Best and Solandt, 1940) although several workers have shown that certain of the circulatory changes in the two conditions are not comparable (Blalock and Levy, 1937; and others). Many of the studies on histamine shock have been carried out upon anesthetized animals. It is known, however, that anesthetics may influence markedly the effects of histamine (Dale and Laidlaw, 1918-19; Dale, 1920). This investigation was undertaken to study some of the changes produced by histamine in dogs without the use of anesthesia. The results were compared with data obtained in this laboratory on traumatic shock in unanesthetized dogs (Gregersen and Root, unpublished experiments).

METHODS. Experiments were carried out on adult mongrel dogs ranging in size from 6.0 to 13.9 kgm. Studies on the circulatory effects of histamine in eighteen normal dogs were supplemented with nine experiments on splenectomized dogs. Splenectomies were performed aseptically upon animals anesthetized with nembutal. Histamine was injected from two days to more than a year after splenectomy. In addition, six dogs anesthetized with ether were subjected to histamine shock. Ether was administered continuously for two hours before the injection of histamine.

Histamine diphosphate was injected subcutaneously³ in the thigh. Histamine dosages were of the order of 10 mgm. of histamine base per kgm. (7.2 to 11.5 mgm. per kgm.) except in the experiments on splenectomized dogs in which

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³ Preliminary experiments showed that it was not practical to administer large doses of histamine intravenously to unanesthetized dogs because of the variability of the symptoms produced, and consequent difficulty of predicting an adequate but not excessive shock dose. A similar difficulty has been noted by other investigators (Smith, 1928; Neuwelt, Levinson and Necheles, 1941).

about 3 mgm. per kgm. (2.6 to 4.7 mgm. per kgm.) was injected. Solutions of histamine diphosphate in 0.9 per cent NaCl were made up fresh for each experiment, with the volume of solution usually 10 cc. The concentrations of histamine ranged between 2 and 14 mgm. histamine base per cubic centimeter, but no correlation was found between the concentration of histamine injected and the symptoms produced.

The femoral vein and artery were exposed under local procaine (2 per cent) anesthesia for blood sampling and injections. After a control period of $1\frac{1}{2}$ or more hours, histamine was injected and the animal observed during the following 2 to $7\frac{1}{2}$ hours. Mean arterial pressure measurements with a mercury manometer were made by direct puncture of the femoral artery. The heart rate was counted with a stethoscope. Rectal temperatures were also taken. In some experiments, venous pressures were measured with a saline manometer in the femoral vein; apparent central venous pressure measurements were obtained with a catheter passed through the left external jugular vein into the atrium.

Plasma volume measurements were made with the dye dilution method, using the dye T-1824 and the technique described by Gregersen and Stewart (1939). In most of the experiments the dye was injected into the femoral vein. Arterial samples were obtained for determination of the serum dye concentration with a K  nig-Martens visual spectrophotometer. All values of plasma volume were calculated from semilog plots (Gregersen and Rawson, 1943). The protein concentration of each sample was determined refractometrically (Neuhausen and Rioch, 1923) with an Abbe refractometer. When, during the course of a dye curve the protein concentration varied by more than 0.2 gram per cent from the control value, dye concentrations were corrected with reference to the control protein concentration. The precision measure of the plasma volume determination was estimated to be ± 3 per cent.

In two experiments, the serum protein concentrations determined refractometrically were compared with those obtained using the Kjeldahl method.

The hematocrit values were measured by centrifuging heparinized arterial blood samples in Wintrobe tubes for 30 minutes at 3000 r.p.m. (radius 13 cm.). Hemoglobin concentrations were measured spectrophotometrically (Drabkin and Austin, 1932) in five experiments on three splenectomized dogs.

Blood and red cell volumes were calculated from the relations:

$$\text{Blood Volume} = \frac{\text{Plasma volume} \times 100}{100 - 0.96 \times \text{Hematocrit}}$$

$$\text{Cell Volume} = \text{Blood Volume} - \text{Plasma Volume}$$

The factor 0.96 was used to correct the hematocrit values for the amount of plasma trapped among the red cells on centrifuging (Gregersen and Schiro, 1938). No attempt was made to correct for the possible differential distribution of red cells between the large and small blood vessels (Fahraeus, 1929). Under the conditions of this series of experiments, the precision measure of the calculated changes in blood and red cell volumes was of the order of ± 10 per cent.

RESULTS. Symptoms of poisoning appeared in less than five minutes after histamine injection. The arterial pressure fell precipitously, the heart rate increased, and respiration was depressed. Lacrimation, salivation, temporary pupillary dilatation and protrusion of the nictitating membrane were frequently noted. In the unanesthetized dogs, defecation occurred and continued intermittently throughout the course of the experiment. Vomiting was rarely seen in the first half-hour after the injection, but often occurred one or two hours later.

The skin and mucous membranes of the unanesthetized animals were usually pale and cyanotic for one-half to two hours after the injection. Gradually the skin and mucous membranes became flushed and hot and the eyes became markedly bloodshot. In two of the three animals showing early flushing, the blood pressure was unusually high. This was also true of the etherized dogs, in which early flushing was noted more frequently. The rectal temperature change was variable (-2.7° to 1.4°C.) in the unanesthetized dogs; the temperature usually fell after histamine in the dogs under ether anesthesia.

The appearance of the unanesthetized animals injected with histamine was characteristic. Instead of showing the apathy of traumatic shock, these animals usually were alert and responsive to external stimuli. Although the arterial pressure was greatly reduced, the dogs were capable of considerable activity. Four animals removed from the board when their arterial pressures were between 30 and 50 mm. Hg were found to be able to stand and walk. They showed interest in their surroundings, and drank when offered water. Thirst was a characteristic symptom of histamine poisoning.

Of the eighteen normal dogs injected with histamine, six died in three to twenty-four hours. All of the splenectomized dogs survived the smaller histamine doses administered. Four of the etherized dogs died in four to six hours.

Changes in mean arterial pressure. The changes in arterial pressure after histamine are shown in figure 1. The blood pressure began to decrease 1 to 2 minutes after the injection, reaching a minimum in 5 to 20 minutes. In the normal unanesthetized dogs the lowest pressures reached were 25 to 44 mm. Hg. Usually the pressure remained about 35 mm. Hg for more than half an hour. In the animals which survived, the pressure gradually increased toward the control value in the following 5 to 6 hours. The same trend was seen in the splenectomized dogs except that the pressure often fell to a lower value and rose less rapidly. The minimum pressures ranged between 17 and 57 mm. Hg. Some of the dogs maintained a pressure under 35 mm. Hg for more than 2 hours. Since the splenectomized dogs received histamine dosages of the order to 3 mgm. per kgm., or one-half to one-third the amount given to normal dogs, this would indicate that the splenectomized dogs were less resistant to the hypotensive effects of histamine than were normal animals. Blood pressure in a normal dog receiving 3 mgm. per kgm. fell from a control level of 100 to 68 mm. Hg. Within two hours the blood pressure had risen to 86.

The fall in blood pressure in the etherized dogs was less severe than in most of the unanesthetized animals. The minimum pressures noted were 45 to 60 mm. Hg, and the pressures remained close to or above 50 during the first hour. In

one experiment the pressure rose from a minimum of 60 and was maintained for about an hour at 70 to 80 mm. Hg. During the second and third hours after histamine injection the pressure fell, but was still above 50 in four of the six experiments. The two animals which survived longest had blood pressures above 40 mm. Hg five to six hours after histamine. In a control experiment in which ether was administered for $7\frac{1}{2}$ hours without the injection of histamine the blood pressure varied only slightly about 110 mm. Hg.

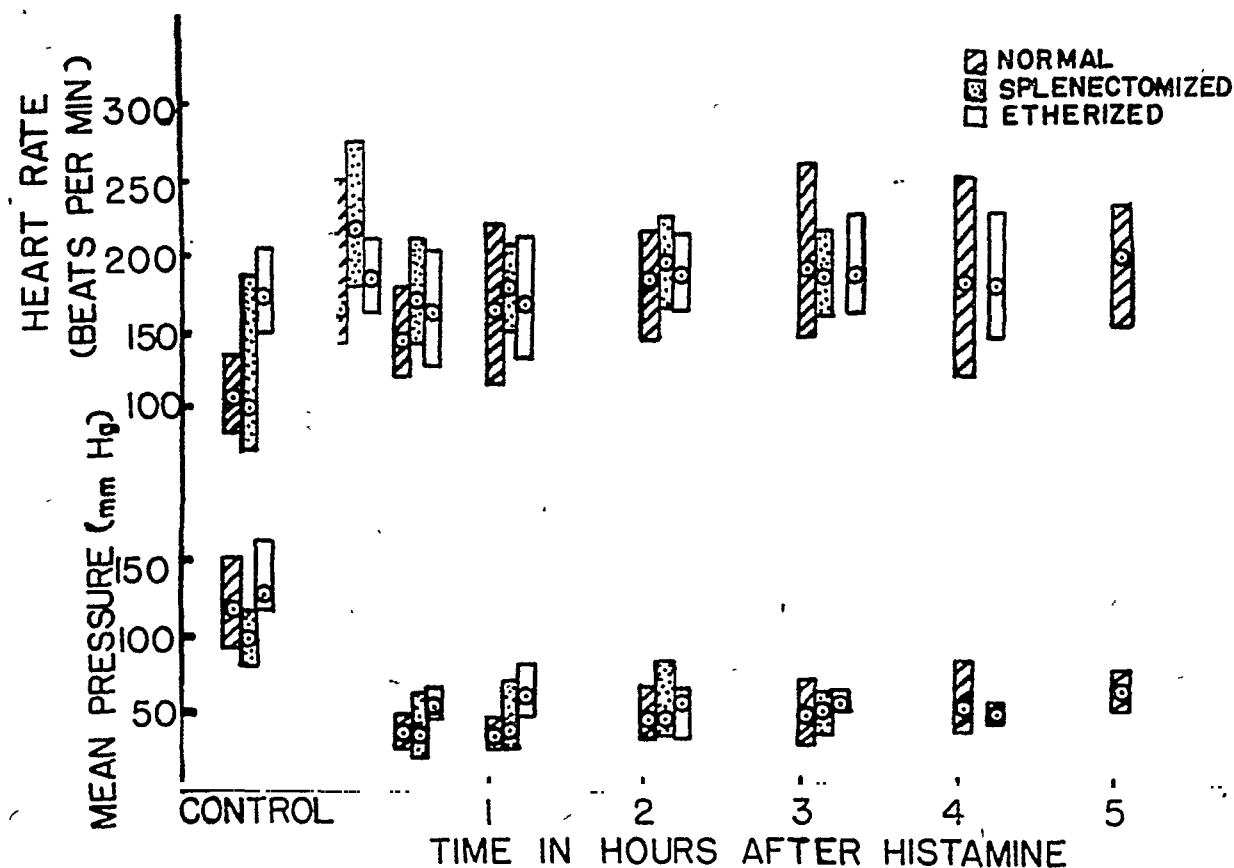


Fig. 1. Showing changes in heart rate and blood pressure after subcutaneous histamine injection in 18 experiments on normal dogs, 9 experiments on splenectomized dogs, and 6 experiments on etherized dogs.

The average values are indicated by circles, and the range of blood pressures and heart rates observed is shown by the height of the columns.

Changes in heart rate, cardiac output and venous pressure. An immediate effect of the histamine injection in unanesthetized dogs (normal and splenectomized) was a sudden increase in heart rate (fig. 1). The heart rate then became irregular, fell from its initial peak and subsequently rose steadily towards 200 beats per minute during the 4 to 5 hours succeeding the injection. Usually the rate did not exceed 212 beats per minute, but in some experiments rates as high as 257 to 268 were recorded.

After histamine injection, the heart beat became weak or even inaudible, as well as more rapid. In a series of seven experiments in collaboration with Doctors

Gregersen, Root and Walcott, cardiac output determinations (Fick method) were made, and the results may be cited briefly. The cardiac output was greatly reduced in the first half-hour after histamine, falling to 8 to 40 per cent of the control value. It usually increased somewhat during the next 2 to 4 hours, but as the heart rate rose progressively the stroke volume did not increase so much as the minute output. In general, changes in cardiac output seemed to be roughly correlated with both the mean arterial pressure and the clinical condition of the animal.

The dogs under ether anesthesia had initially elevated heart rates of 140 to 204 beats per minute. Usually after a slight rise immediately following the injection, the heart rate fell off during the next 30 to 60 minutes and later rose gradually. The heart rates were thus of the same order as the rates in the unanesthetized dogs, although they had been higher before histamine injection. In contrast to the finding after histamine in unanesthetized dogs, the sound of the beat was usually fairly strong throughout the early post-histamine period.

During the first hour after histamine injection the central venous pressure fell in 7 normal unanesthetized dogs by 13 to 40 mm. saline, and more markedly (by more than 80 mm. saline) in 2 etherized dogs. Subsequently the central venous pressure fell further or rose only slightly, so that at the end of the experiment it was 10 to 40 mm. saline below the control level.

In 5 unanesthetized or etherized dogs, the femoral venous pressure varied inconstantly about the control value, falling shortly after the injection by 9 to 35 mm. saline in four, and rising 8 mm. in one. As the blood pressure rose, femoral venous pressure also tended to rise towards or above the control level. In many of the experiments the femoral veins appeared well filled, so that blood could be withdrawn from them without difficulty.

Changes in plasma volume and serum protein concentration. Histamine, injected during the later part of the control T-1824 time-concentration curves, produced varying changes in the curves. Frequently the slopes of the curves increased, but when corrections were made for simultaneous changes in serum protein concentrations, the curves often resumed their pre-histamine slopes. Reinjections of dye $\frac{1}{2}$ hour or longer after histamine injection showed that, in general, the character of the dye curves was not markedly altered by histamine (fig. 2). Although the apparent mixing time frequently increased and the rate of dye disappearance decreased after histamine injection, these differences between pre- and post-histamine dye curves were not significant statistically.

The measured changes in plasma volume are shown in table 1. During the first $\frac{1}{2}$ to $1\frac{1}{2}$ hours after histamine, when the blood pressure was lowest, the plasma volume in about half the experiments on unanesthetized dogs was unchanged or increased. Later it decreased. It was reduced in all the etherized dogs.

After histamine, the serum protein concentration showed a slight to marked decrease in 11 of the 18 normal dogs, in 6 of the 9 splenectomized dogs, and in 3 of the 6 etherized dogs. Changes in calculated total circulating serum protein (serum protein concentration \times plasma volume) are shown in table 2. In two experiments in which the serum protein concentrations were simultaneously deter-

mined by the refractometric and Kjeldahl methods, the serum protein concentrations varied within ± 3 to 4 per cent of the control level as determined by both methods.

Changes in hematocrit value, hemoglobin concentration and calculated red cell and blood volumes. The injection of histamine usually resulted in a marked rise in hematocrit value in the normal dogs. Frequently as the experiment proceeded, the hematocrit reading continued to rise slowly. The initial rise occurred rapidly (fig. 3), and was not accompanied by a corresponding decrease in plasma volume or increase in serum protein or dye concentration. This rise might be attributed to an increase in number of circulating cells resulting from contraction of the

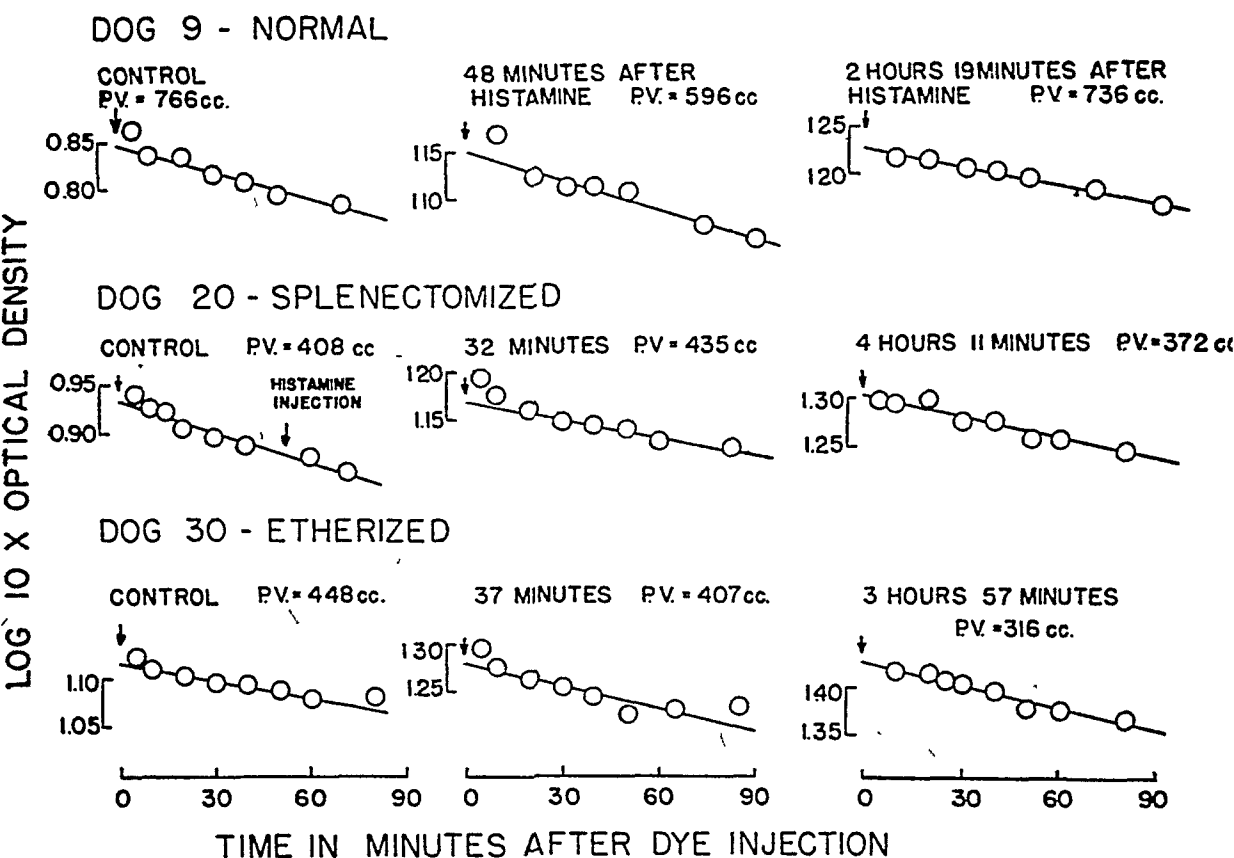


Fig. 2. Typical T-1824 time-concentration curves before and after histamine injection. Times after histamine injection, and calculated plasma volumes, are indicated.

spleen, but it was found to be present, though less marked, in 5 dogs which had been splenectomized 20 days to more than a year before histamine injection. An appreciable rise in hematocrit reading also occurred in half the etherized dogs. Change in the hematocrit value was slight in 3 normal and 3 etherized dogs, and in 4 dogs subjected to histamine injection 2 to 21 days after splenectomy.

The measured rise in hematocrit value was not caused by sudden change in mean red cell volume since in 5 experiments on 3 splenectomized dogs the increase in hemoglobin concentration was usually equal to or greater than the rise in the hematocrit values.

TABLE 1

Showing the effects of subcutaneous histamine injection on the plasma volume of dogs

DOG NUMBER	BODY WEIGHT	CONTROL VOLUME	PLASMA VOLUME				COMMENTS
			$\frac{1}{2}$ to 1 $\frac{1}{2}$ hours after histamine		2 to 5 hours after histamine		
			Volume	Change	Volume	Change	
	kgm.	cc.	cc.	per cent	cc.	per cent	
1	8.77	429	522	21.7			Normal
2	7.7	552	536	-2.1			Normal
3	12.22	681	755	10.9			Normal
4	7.83	456	450	-1.3			Normal
5	7.62	442	398	-10.0			Normal
6	8.65	434	374	-13.8	416	-4.3	Normal
7	9.15	696	619	-11.1	524	-24.7	Normal
8	7.88	372	455	22.3			Normal
9	13.86	766	596	-22.2	736	-3.9	Normal
10	8.64	466	399	-20.8	344	-26.2	Normal
11	7.10	419	441	5.2			Normal
12	6.50	390	456	16.9	343	-12.0	Normal
13	7.45	370	403	8.9			Normal
14	12.51	528	648	22.7	514	-2.6	Normal
15	10.45	608	605	-0.5	507	-16.6	Normal
16	9.74	466			559	-20.0	Normal
17	9.15	420	455	8.3			Normal
18	6.49	354	328	-7.3			Normal
Averages.....				1.6		-13.8	
19	10.22	536	540	0.7			Splenect.— 1 year*
20	7.36	408	435	6.6	372	-8.8	Splenect.— 1 year*
21	7.25	496	529	6.6	492	-0.8	Splenect.— 20 days*
21	7.07	490	460	-6.1	412	-15.9	Splenect.— 31 days*
22	8.39	510	512	-0.4			Splenect.— 21 days*
22	9.04	485	517	6.6			Splenect.— 72 days*
23	8.89	715	755	5.6	740	3.5	Splenect.— 5 days*
24	8.95	450	400	-11.1			Splenect.— 2 days*
25	9.71	484			401	-17.2	Splenect.— 3 days*
Averages.....				1.1		-7.8	
26	9.13	417†	400	-4.1			Etherized
27	8.2	396†	318	-19.7			Etherized
28	10.92	688†	530	-23.0			Etherized
29	9.46	455†	374	-17.8	394	-13.4	Etherized
30	7.2	448†	407	-9.2	316	-29.5	Etherized
31	11.47	509†	481	-5.5	432	-15.1	Etherized
Averages.....				-13.2		-19.3	

* Time between splenectomy and experiment.

† Plasma volume determined after one hour of anesthesia.

TABLE 2

Showing the effect of subcutaneous histamine injection on calculated total circulating protein (plasma volume \times serum protein concentration) of dogs

DOG NUMBER	CONTROL AMOUNT	TOTAL CIRCULATING PROTEIN				COMMENTS
		$\frac{1}{2}$ to $1\frac{1}{2}$ hours after histamine		2 to 5 hours after histamine		
		Amount	Change	Amount	Change	
	gm.	gm.	per cent	gm.	per cent	
1	24.7	27.7	12.1			Normal
2	30.4	27.3	-10.2			Normal
3	34.7	35.5	2.3			Normal
4	21.4	21.2	-0.9			Normal
5	28.1	21.5	-23.5			Normal
6	26.5	24.0	-9.4	30.2	14.0	Normal
7	36.5	32.2	-11.8	29.1	-20.3	Normal
8	20.3	22.1	8.8			Normal
9	44.8	41.4	-7.6	43.0	-4.0	Normal
10	25.2	21.3	-15.4	18.9	-25.0	Normal
11	22.0	20.9	-5.0			Normal
12	20.9	19.8	-5.3	16.5	-21.0	Normal
13	19.6	19.9	-1.5			Normal
14	30.9	34.7	12.3	27.5	-11.0	Normal
15	35.0	32.4	-7.4	27.1	-22.6	Normal
16	24.7			32.2	30.3	Normal
17	25.0	24.6	-1.6			Normal
18	17.9	16.4	-8.4			Normal
Averages.....			-4.3		-7.4	
19	40.8	39.8	-2.0			Splenectomized
20	23.7	23.8	0.4	23.2	-2.1	Splenectomized
21	27.0	28.3	4.8	27.6	2.2	Splenectomized
21	29.2	27.0	-7.5	26.2	-10.3	Splenectomized
22	30.4	28.5	-6.2			Splenectomized
22	27.9	26.9	-3.6			Splenectomized
23	28.4	27.0	-4.9	27.3	-3.9	Splenectomized
24	24.5	21.6	-11.8			Splenectomized
25	31.0			28.0	-9.7	Splenectomized
Averages.....			-3.8		-4.8	
26	30.7*	29.5	-3.9			Etherized
27	25.0*	18.8	-24.8			Etherized
28	37.5*	30.0	-20.0			Etherized
29	26.7*	21.9	-18.0	23.6	-11.6	Etherized
30	29.2*	26.3	-9.9	21.7	-25.6	Etherized
31	32.4*	30.9	-4.6	27.0	-16.7	Etherized
Averages.....			-13.5		-18.0	

* Total protein determined one hour after induction of anesthesia.

Calculated changes in total circulating blood and red cell-volumes are shown in tables 3 and 4.

The necropsy findings in unanesthetized and anesthetized dogs were similar. The abdominal and pelvic viscera appeared much congested and the thoracic and abdominal cavities were moist and occasionally contained free fluid. The spleen was moderately contracted. Hemorrhages in the left ventricular myocardium were seen frequently, and were particularly severe in the etherized dogs. In the gastro-intestinal tract, hemorrhages were found most often in the colon,

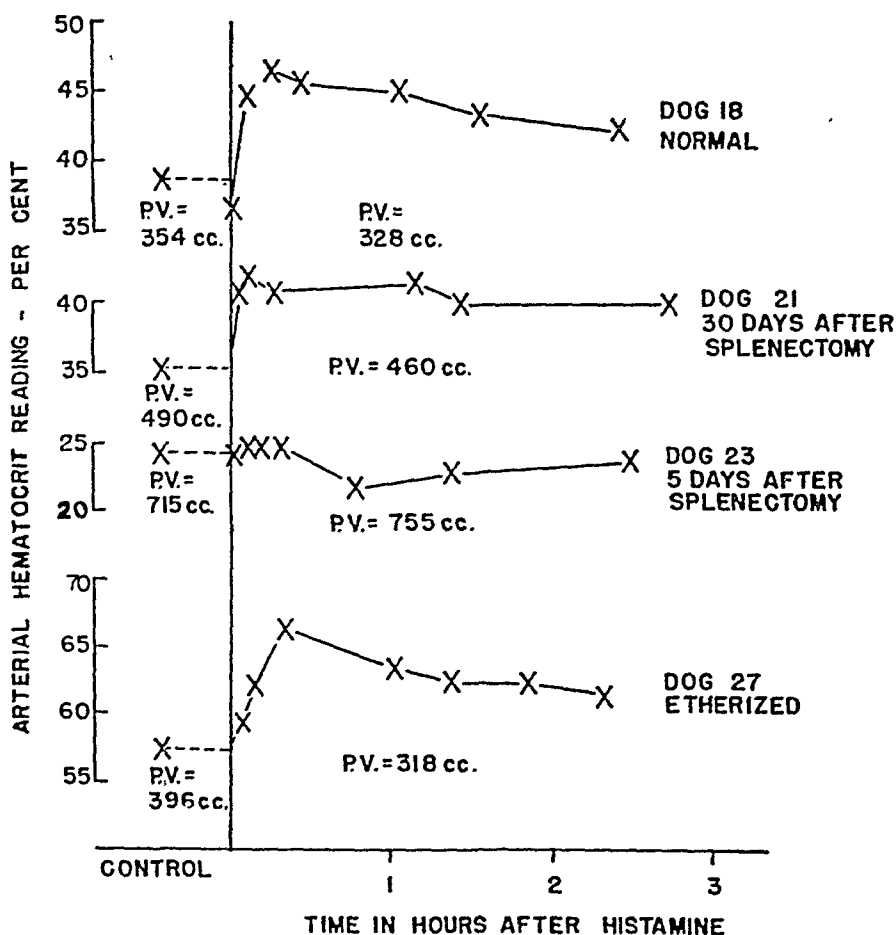


Fig. 3. Showing characteristic changes in hematocrit readings following histamine injection. Plasma volume measurements before and after histamine injection are indicated.

but also occasionally in the stomach, duodenum, pancreas and serosa of the gall bladder. Rarely the adrenal glands, mesenteric lymph nodes and lungs were hemorrhagic. The thoracic duct was filled with bloody, dye-stained lymph.

The stomach often contained large amounts (100 to 200 cc.) of fluid, the amount probably depending in part on the frequency with which vomiting had occurred. Usually the bladder was contracted and empty—a finding which is of interest since, in conjunction with the fact that urination rarely occurred during the course of the experiment, it indicates that urine formation had been suspended during the histamine hypotension.

TABLE 3

Showing the effect of histamine injection on the calculated total circulating blood volume of dogs

DOG NUMBER	CONTROL VOLUME	TOTAL CIRCULATING BLOOD VOLUME				COMMENTS
		$\frac{1}{2}$ to 1½ hours after histamine		2 to 5 hours after histamine		
		Volume	Change	Volume	Change	
	cc.	cc.	per cent	cc.	per cent	
1	867	1152	32.8			Normal
2	922	927	0.5			Normal
3	1097	1327	21.0			Normal
4	779	902	15.8			Normal
5	801	843	5.2			Normal
6	805	947	17.6	1053	30.8	Normal
7	1210	1274	5.3	1245	2.9	Normal
8	684	930	36.0			Normal
9	1350	1290	-4.4	1496	10.8	Normal
10	888	891	0.3	796	-10.4	Normal
11	639	704	10.2			Normal
12	722	884	22.4	728	0.8	Normal
13	705	890	26.2			Normal
14	1086	1565	44.1	1342	23.6	Normal
15	1156	1137	-1.6	1054	-8.8	Normal
16	820			1150	40.2	Normal
17	743	858	15.5			Normal
18	557	578	3.8			Normal
Averages.....			14.7		11.2	
25	866			750	-13.4	Less than 22 days after splenectomy
24	704	643	-8.7			Less than 22 days after splenectomy
23	936	968	3.4	956	2.1	Less than 22 days after splenectomy
22	879	886	0.8			Less than 22 days after splenectomy
21	780	879	12.7	811	4.0	Less than 22 days after splenectomy
22	892	983	10.2			More than 22 days after splenectomy
21	763	759	-0.5	680	-10.9	More than 22 days after splenectomy
20	780	902	15.6	816	4.6	More than 22 days after splenectomy
19	976	1055	8.1			More than 22 days after splenectomy
26	846*	934	10.4			Etherized
27	878*	862	-1.8			Etherized
28	1373*	1130	-17.7			Etherized
29	981*	813	-17.1	842	-14.2	Etherized
30	750*	741	-1.2	620	-17.3	Etherized
31	1052*	1108	5.3	909	-13.6	Etherized
Averages.....			-3.7		-15.0	

* Blood volume determined after one hour of anesthesia.

TABLE 4

Showing the effect of histamine injection on the calculated total circulating red cell volume of dogs

DOG NUMBER	CONTROL VOLUME	TOTAL CIRCULATING RED CELL VOLUME				COMMENTS
		$\frac{1}{2}$ to $1\frac{1}{2}$ hours after histamine		2 to 5 hours after histamine		
		Volume	Change	Volume	Change	
	cc.	cc.	per cent	cc.	per cent	
1	438	630	43.8			Normal
2	370	391	5.7			Normal
3	416	572	37.5			Normal
4	323	452	40.0			Normal
5	359	445	24.0			Normal
6	371	573	54.4	637	71.7	Normal
7	514	655	27.4	721	40.3	Normal
8	312	475	52.2			Normal
9	584	594	1.7	760	30.2	Normal
10	422	522	23.7	452	7.1	Normal
11	220	263	19.5			Normal
12	332	428	28.9	385	16.0	Normal
13	335	487	45.4			Normal
14	558	917	64.3	828	48.4	Normal
15	548	532	-2.9	547	0.2	Normal
16	354			591	66.0	Normal
17	323	403	24.8			Normal
18	203	250	23.2			Normal
Averages.....			30.2		35.0	
25	382			349	-8.6	Less than 22 days after splenectomy
24	254	243	-4.3			Less than 22 days after splenectomy
23	221	213	-4.1	216	-2.3	Less than 22 days after splenectomy
22	369	376	1.9			Less than 22 days after splenectomy
21	284	350	23.8	319	12.3	Less than 22 days after splenectomy
22	407	466	14.5			More than 22 days after splenectomy
21	273	299	9.5	268	-1.8	More than 22 days after splenectomy
20	372	467	25.5	444	19.4	More than 22 days after splenectomy
19	440	515	17.0			More than 22 days after splenectomy
26	429*	534	24.5			Etherized
27	482*	544	18.9			Etherized
28	685*	600	-12.4			Etherized
29	526*	439	-16.5	448	-14.8	Etherized
30	302*	334	10.6	304	0.7	Etherized
31	543*	627	15.5	477	-12.2	Etherized
Averages.....			5.8		-8.8	

* Red cell volume determined after one hour of anesthesia.

In general, the organs of the unanesthetized dogs were not grossly stained with T-1824. In one instance, the gastro-intestinal tract appeared to be diffusely stained, and this was a constant finding in the etherized dogs. Staining of the arch of the aorta and the A.V. and semilunar valves of the heart was nearly always observed. Less frequently the adrenals and mesenteric lymph nodes were stained with T-1824 in both the normal and etherized dogs. Dye was never found at the site of the subcutaneous injection of histamine.

DISCUSSION. A characteristic state of profound circulatory disturbance was produced rapidly by subcutaneous histamine injection. The survival time of the normal dogs was longer than the survival time of the etherized animals. Yet the fall in mean arterial pressure resulting from equal amounts of histamine was less severe in the dogs under ether anesthesia. This finding was unexpected in view of the reported decrease in resistance of etherized dogs and cats to intravenously injected histamine (Dale and Laidlaw, 1918-19). Favorable circulatory changes produced by ether anesthesia (e.g., elevation of the cardiac output. Blalock, 1927) may perhaps act initially to antagonize the hypotensive effects of histamine.

The maintenance of mean arterial pressure at normal levels depends on mutual adjustment between the peripheral resistance and cardiac output. Various investigators have attributed the hypotensive action of histamine in dogs and cats to changes in both of these factors. In dogs, histamine has been shown to dilate arterioles (Burn and Dale, 1926). This would tend to reduce peripheral resistance even if arterial constriction occurred simultaneously (Burn and Dale; and others), since it is recognized that variations in peripheral resistance are largely determined by the caliber of the arterioles. Cardiac output may be decreased by a specific depressant action of histamine on the heart (Rühl, 1929; Dixon and Hoyle, 1930); or by marked reduction in venous return caused by general vasodilatation (Dale and Laidlaw, 1910), decrease of circulating blood volume (Dale and Laidlaw, 1918-19; Smith, 1928; Eppinger and Schürmeyer, 1928; and others), or by damming back of blood in the portal-hepatic system as the result of constriction of the hepatic veins (Mautner and Pick, 1922). It is possible that under certain conditions a combination of these changes may act together to cause the symptoms resulting from histamine injection. In the present series of experiments, the rapidity and profoundness of the fall in mean arterial pressure to a level which was often below the estimated upper limits of normal capillary pressure (30-35 mm. Hg) suggests arteriolar dilatation. Total peripheral resistance, calculated from the ratio of mean pressure to cardiac output, increased in most of the 7 experiments in which it was measured. Yet this cannot be taken as conclusive evidence against arteriolar dilatation. The effect of arteriolar dilatation on calculated total peripheral resistance might be masked by simultaneous changes acting in the opposite direction, such as increase in blood viscosity resulting from rise in red cell concentration. Constriction of the hepatic veins would probably act in the same way, but no evidence was obtained as to whether this occurred. Heart damage probably was not the primary cause of the hypotension. With other factors unchanged, myocardial weakening

sufficient to cause the observed fall in blood pressure should be accompanied by a marked rise in venous pressure. Yet apparent central venous pressure fell after subcutaneous histamine injection. Vasodilatation, which may have involved all the smaller blood vessels at least in the later stage of shock, was indicated by congestion of the viscera and flushing. Marked reduction in blood volume was not present.

The observation that the plasma volume was increased or only moderately reduced early in histamine shock is not in agreement with the usual finding of marked reduction in plasma volume after intravenous histamine injection (Smith, 1928; Butler, Beard and Blalock, 1931; and others). On the other hand, Hueper and Ichniowski (1943) noted little change, and often reduction, in hematocrit measurements in dogs under nembutal anesthesia injected subcutaneously with histamine suspended in an oil-lanolin medium. They inferred that hemodilution had occurred.

The change in plasma volume might be attributed to one or a combination of histamine effects. The stimulation of gastro-intestinal secretion would tend to reduce plasma volume. Probably more important are changes in the conditions regulating capillary fluid exchange. *a.* Depending on the degree of arteriolar dilatation, the profound fall in mean arterial pressure must be reflected by a fall in capillary pressure. If other factors remained constant, reduction in average capillary pressure would favor absorption of fluid and increase in plasma volume. *b.* It has been suggested that histamine acts directly on the capillary endothelium to increase its permeability to large molecules (Dale, 1920; and others), thus effectively decreasing the colloid osmotic pressure of the blood. If this occurred, it would throw the balance of capillary fluid exchange toward the negative side, and plasma volume should decrease. In this series of experiments there was no evidence for increased capillary permeability to protein. The rate of escape of the albumin-bound dye T-1824 from the circulation, estimated from time-concentration curves and staining of the tissues, did not appear to increase significantly. Calculated total serum protein tended to decrease, but this probably should not be taken as evidence for increased permeability of the capillaries to protein without more knowledge of the mechanism maintaining the constancy of the protein content of the plasma. In this mechanism the relative impermeability of the capillary endothelium to proteins is probably only one factor. Changes induced by subcutaneous histamine in other factors—e.g. lymph protein transfer and the destruction and synthesis of plasma protein—are not known.

In normal dogs the rise in hematocrit values over and above corresponding decrease in plasma volume, and the consequent increase in calculated total circulating red cell volume might result from 1, mobilization of red cells from blood depots of relatively high corpuscular content; 2, change in the differential distribution of red cells between the large and small blood vessels (Fahraeus, 1929); or 3, decrease in plasma volume, unmeasured because of systematic errors in the dye dilution technique inherent in its use in the condition of histamine poisoning.

Against the last possibility is the finding that the character of the dye curves was not appreciably altered by histamine. Either of the last two possibilities should apply equally to normal and splenectomized dogs, and in addition to cats as well as to dogs. Yet when histamine was injected subcutaneously in 4 dogs less than three weeks after splenectomy, the marked rise in hematocrit value did not occur. In addition, the increase in hematocrit reading was not observed in 6 preliminary experiments on normal and anesthetized cats injected subcutaneously with histamine.⁴ These findings would be compatible with the view that histamine acted to mobilize red cells from the spleen—and possibly other blood depots—in normal dogs and in dogs fully recovered from splenectomy. In dogs under ether anesthesia, the discrepancy between rise in hematocrit value and change in plasma volume was less than in many of the normal dogs, a finding which would be in agreement with the possibility that histamine mobilizes cells from depots, the major one of which—the spleen—was already partially emptied in etherized animals by the action of the anesthetic. A parallel is suggested between the effects of histamine and of ether, which constricts the spleen and thereby raises the hematocrit value in dogs but not in cats (McAllister, 1938; Conley, 1941).

The effects of subcutaneous histamine injection seen in these experiments were in marked contrast to the symptoms of traumatic shock in unanesthetized dogs. The animals in histamine shock were more active and alert, in spite of their much greater hypotension. This difference might result from cerebral vasodilatation, with consequent maintenance of blood flow to the brain, which is said to be one effect of histamine in dogs (Weinberg, 1933). Reduction in blood volume was not produced by histamine. This was perhaps the most fundamental of the observed differences between histamine shock, as produced under the conditions of these experiments, and traumatic shock.

SUMMARY

Subcutaneous injection of 3 to 12 mgm. histamine base per kgm. in unanesthetized and etherized dogs resulted in a characteristic circulatory disturbance which differed markedly from traumatic shock in the clinical symptoms produced, in the far greater hypotension, and in the absence of definite blood volume reduction as an etiological factor. Change in blood volume in histamine shock under these conditions resulted from 1, a moderate increase or decrease in plasma volume, and 2, increase in calculated red cell volume, which may have resulted from mobilization of cells from the spleen and other blood depots.

⁴ Dale and Laidlaw (1918-19) noted a pronounced rise in hematocrit, with fall in serum protein concentration, in etherized cats injected intravenously with histamine. This finding, which is in contrast to the finding in cats injected subcutaneously with histamine, was confirmed in two experiments in which shock was produced in etherized cats in the manner described by Dale and Laidlaw. This difference between the effects of intravenous and subcutaneous histamine in cats is unexplained. It serves to emphasize the fact that histamine shock may not only vary markedly from species to species, but also within a given species depending on the conditions under which it was produced.

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REFERENCES

- BEST, C. H. AND D. Y. SOLANDT. *Brit. Med. J.* 799, 1940.
BLALOCK, A. *Arch. Surg.* 14: 732, 1927.
BLALOCK, A. AND S. E. LEVY. *This Journal* 118: 734, 1937.
BURN, J. H. AND H. H. DALE. *J. Physiol.* 61: 185, 1926.
BUTLER, V., J. W. BEARD AND A. BLALOCK. *Arch. Surg.* 23: 848, 1931.
CONLEY, C. L. *This Journal* 132: 796, 1941.
DALE, H. H. *Brit. J. Exper. Path.* 1: 103, 1920.
DALE, H. H. AND P. P. LAIDLAW. *J. Physiol.* 41: 318, 1910.
J. Physiol. 52: 355, 1918-19.
DALE, H. H., P. P. LAIDLAW AND A. N. RICHARDS. *Med. Res. Council Report* no. 26, 8, 1919.
DIXON, W. E. AND J. C. HOYLE. *J. Physiol.* 70: 1, 1930.
DRABKIN, D. L. AND J. H. AUSTIN. *J. Biol. Chem.* 98: 719, 1932.
EPPINGER, H. AND A. SCHÜRMEYER. *Klin. Wchnscher.* 7: 777, 1928.
FAHRAEUS, R. *Physiol. Rev.* 9: 241, 262, 1929.
GREGERSEN, M. I. AND R. A. RAWSON. *This Journal* 138: 698, 1943.
GREGERSEN, M. I. AND H. SCHIRO. *This Journal* 121: 284, 1938.
GREGERSEN, M. I. AND J. D. STEWART. *This Journal* 125: 142, 1939.
HUEPER, W. C. AND C. T. ICHNIOWSKI. *J. Pharmacol. and Exper. Therap.* 78: 127, 1943.
MCALLISTER, F. F. *This Journal* 124: 391, 1938.
MAUTNER, H. AND E. P. PICK. *Biochem. Ztschr.* 127: 72, 1922.
NEUHAUSEN, B. S. AND D. M. RIOCH. *J. Biol. Chem.* 55: 353, 1923.
NEUWELT, F., S. O. LEVINSON AND H. NECHELES. *Surg.* 9: 503, 1941.
RÜHL, A. *Arch. f. exper. Path. u. Pharmacol.* 145: 255, 1929.
SMITH, M. I. *J. Pharmacol. and Exper. Therap.* 32: 465, 1928.
WEINBERG, S. J. *J. Pharmacol. and Exper. Therap.* 47: 95, 1933.

STUDIES ON THE MECHANISM OF COBALT POLYCYTHEMIA¹

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Although it is perhaps generally recognized that the mechanism of cobalt polycythemia is not completely understood, the work most frequently cited is that of Barron and Barron (1) to the effect that a small amount of cobalt inhibits the respiration, *in vitro*, of various tissues but notably reticulocytes and bone marrow. These authors propose that cobalt polycythemia is due "to inhibition by cobalt of the respiratory function of immature red cells. Once these cells have lost their ability to respire, they are thrown into the circulation as mature non-respiring cells, being replaced in the bone marrow by new cells". Barron and Barron's own data (their table 1) show, however, that the reticulocytes and erythroblasts that appear in the bloodstream in cobalt polycythemia are actively-respiring cells. It has been shown from other studies, however, that cobalt produces increased erythropoietic activity with erythroid metaplasia in the marrow (2,3,4) and a reticulocytosis and polycythemia in the circulating blood (5,6). It is rather difficult to understand how this increased activity of the marrow could occur in the face of impaired respiratory activity, particularly since Warren (7) found that erythroid marrow cells are characterized metabolically by active respiratory and low glycolytic activity. If respiration is impaired, how could the cells undergo more rapid maturation and growth than normally; would they obtain the required energy from increased glycolytic activity? It was largely with these questions in mind that the present study was undertaken but we have also investigated several other possible modes of action of cobalt. Unfortunately, the results in each instance are essentially negative, but it is our hope that the findings to be presented will clarify the work in this field.

A. *Marrow Respiration and Glycolysis in Cobalt Polycythemia.* 1. *The effect on marrow respiration of adding cobalt in vitro.* These experiments are essentially a repetition of those of Barron and Barron (1). Solutions of CoSO_4 have been tipped onto slices of rabbit bone marrow while the respiration was being measured in the Warburg apparatus. Fifteen experiments have been carried out, with the final concentration of cobalt in the vessels varying from 10^{-4} to 10^{-2} molar. Various media have been employed,—neutralized serum (8), saline and Ringer solution, all with and without added phosphate and glucose. Marrows from normal and from cobalt polycythemic animals have been employed, and in other experiments suspensions of rabbit red cells rich in reticulocytes and suspensions of nucleated red cells of ducks have been used. In no instance was an appreciable effect of cobalt observed during the 3-hour experimental period following its addition unless the concentration was as high as 0.01 M when depressions of

¹ A preliminary report of these studies has appeared in *Federation Proceedings* 2:43, 1943.

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respiration of the order of 20 per cent were obtained. Such concentrations are clearly beyond the limit of any which may be encountered in the body using the small amounts required to induce polycythemia (9) and considering its rapid excretion (10). Furthermore, analyses of the marrow and serum of cobalt polycythemic animals kindly performed by Dr. Otto H. Müller with the dropping mercury electrode method (11) revealed the presence of only traces of cobalt. We have taken pains in the respiration experiments, however, to neutralize the cobalt solutions, which are very acid, to about pH 6.5 (precipitates form at higher pH's) and to discard experiments in which gross clumping of the marrow slices occurred in the presence of cobalt. These possible sources of error may conceivably have contributed to the results reported by Barron and Barron.

2. *Respiration and glycolysis of marrow of cobalt-polycythemic rabbits.* A more direct test of the theory of Barron and Barron is the determination of the rate of respiration of the marrow of cobalt-polycythemic animals. This measurement was carried out on the marrows of 15 rabbits at intervals of from 10 to 81 days after the start of cobalt administration. All the animals showed some degree of

TABLE 1
Respiration and glycolysis of bone marrow from polycythemic rabbits

	Q_{O_2}	$Q_G^{O_2}$	$Q_G^{N_2}$	$Q_G^{N_2}/Q_{O_2}$
Approximate values expected from (7)	7.0	2.3	13.3	1.9
Corresponding values found	$6.5 \pm 0.2^*$	2.2 ± 0.03	11.7 ± 0.5	1.8

* Probable error of the mean, $\frac{\sqrt{\Sigma d^2}}{n(n-1)}$. Corresponding errors for the reference data in the line above cannot readily be calculated, but the errors are of about the same order of magnitude.

polycythemia, the increase in red cell count varying from 1.3 to 3.9 and averaging 3.6 million cells per cu. mm. The average proportion of erythroid cells in the marrow smears at the time of sacrificing the animals was 58 per cent. The techniques employed have been described previously (8) and respiration and glycolysis values have been given for marrows containing varying proportions of myeloid and erythroid cells (7).

In table 1 the corresponding data obtained on the polycythemic marrows is compared with the data to be expected from the previous study. The two sets of data agree so closely that the difference between them, while just significant statistically, is of very doubtful significance physiologically. This is particularly true of the ratio of anaerobic glycolysis to respiration (last column), which has previously been found to be the most reliable criterion of the metabolic activity of the marrow (7). Metabolic changes may occur in the marrow as the result of cobalt administration, but they do not appear to be reflected by appreciable changes in the overall respiration or glycolysis.

3. *The effect of serum of cobalt-polycythemic animals on marrow respiration.* As a third test of the theory that marrow respiration is impaired in cobalt polycy-

themia, seven experiments were performed in which the respiration of normal marrows was measured both in normal serum and in that from cobalt-polycythemic animals. Respiration in the serum of the polycythemic animals averaged only 1 per cent less than in normal serum, with a P.E. of ± 3.8 per cent. In nine experiments, the reverse determination was carried out, respiration of the marrow from cobalt-polycythemic animals being measured in serum from both normal and polycythemic animals. The rate of respiration averaged 3 per cent lower in the normal serum, with a P.E. of ± 4.6 per cent. Clearly, no significant effect of the sera on either normal or polycythemic marrows could be established.

B. *Possible Neural Mode of Action of Cobalt.* In a series of papers (12-16) Davis has demonstrated that choline and certain other vasodilator drugs depress or prevent the polycythemia following cobalt administration, whereas vasoconstrictor drugs can induce polycythemia. The action of vasodilator drugs in inhibiting cobalt polycythemia is interpreted by Davis in terms of the Barron-and-Barron theory, *i.e.*, the inhibitory action of cobalt on the respiratory activity of the erythroid marrow cells is counteracted by the increased oxygen supply to the marrow resulting from the vasodilatation. Since we cannot confirm the Barron and Barron theory of impaired respiration of the marrow cells, an alternative explanation of the antagonistic action of cobalt and the vasodilator drugs suggested itself, namely, that cobalt might act by a local neural mechanism (vasoconstriction?) that is directly counteracted by choline. If this were true, denervation of the marrow should prevent the effect of cobalt.

This possibility was investigated by inducing cobalt polycythemia in a series of rabbits in which one hind limb was denervated, and making morphological examination of the tibial marrow of the normal and denervated limbs. Eight animals were used in the experiments. The operation consisted of section under local anesthesia of the right femoral nerve as it emerges beneath the inguinal ligament, and of the right sciatic nerve in the upper third of the thigh. At least a centimeter of nerve was removed distal to these points. This procedure destroys both the somatic and autonomic innervation of the right hind leg in most of the thigh; below the knee, the denervation is complete. This was verified by physical examination after the animals had recovered from the operation, and later when they were sacrificed after production of cobalt polycythemia. Complete sensory and motor paralysis was always observed below the level of the knee, and in the later examinations there was marked atrophy of the thigh and leg muscles. The innervation of the vascular supply of the tibial marrow must consequently have been interrupted, for it has been shown that the sympathetic innervation of vessels in the extremities is via the peripheral nerves and that the fibers do not traverse the vessels for more than a short distance (17).

The data on these animals are summarized in table 2. After the operation, cobalt sulfate was administered either orally or subcutaneously in doses of 5 or 7 mgm. cobalt per day together with 3 mgm. of MnCl_2 (18) for periods varying from 19 to 81 days. Usually about 3 weeks were required for the polycythemia to reach its maximum degree, after which it was maintained. The original and

final levels of the red blood cell count and hemoglobin determinations are shown in the table. No difference was noted either in the rate of development or in the degree of polycythemia in the operated animals compared with 7 others in which no operation was performed. When the animals were sacrificed, the gross appearance of the femoral and tibial marrow of the normal and denervated limbs was identical. All the marrows appeared to be hyperplastic but to a variable degree in different animals. This was confirmed by examination of sections prepared from corresponding areas of the tibial marrow on the two sides. The degree of hyperplasia was graded on an arbitrary scale from + to +++++. These gradings are shown in table 2. It is clear that the same extent of erythroid hyperplasia was present in the control and denervated limbs.

TABLE 2

Blood and bone marrow responses following administration of cobalt to animals with a denervated hind limb

EXP. NO.	COBALT DOSAGE	DURATION OF EXP.	RED BLOOD CELL COUNT		HEMOGLOBIN		PER CENT ERY- THROID MARROW CELLS		MARROW HYPERPLASIA	
			Begin- ning	End	Begin- ning	End	Control limb	Denervated limb	Control limb	Denervated limb
	mgm./day	days	Mil.	Mil.	gms. %	gms. %				
6	5	41	5.3	6.8	12.3	15.6	64	61	2+	2+
10	5	61	5.3	8.0	9.0	13.5	59	61	4+	4+
11	5	63	6.2	7.5	7.9	12.5	63	64	3+	3+
12	5	67	5.6	7.1			50	53	2+	2+
13	7	29	4.3	7.8	10.7	16.3	58	59	4+	4+
14	7	81	4.7	7.4	10.4	14.9	59	61	2+	2+
15	7	19	4.2	7.6	9.5	13.7	64	60	4+	4+
16	7	21	5.4	8.0	10.9	13.8	59	60	4+	4+
Average.....			5.1	7.5	10.7	14.3	60	60		

Finally, differential bone marrow counts were made on smears of the tibial marrow of the control and denervated limbs. The proportion of erythroid cells in the marrow of normal rabbits of the same strain is slightly less than 50 per cent (7). In the present series, as shown in table 2, the average proportion of erythroid cells was 60 per cent, both in the control and denervated marrows. This would hardly be a significant increase were it not for the hyperplasia which occurred concomitantly; when these two changes are considered together, it is clear that a definite increase occurred in both the mass and proportion of erythroid cells and that these changes were independent of whether or not the marrow had an intact innervation. We conclude that the morphological response of the bone marrow to cobalt is independent of an intact peripheral innervation.³

C. *Histological Changes in Marrow Blood Vessels.* Reznikoff, Foot and

³ It is also worthy of note that denervation does not affect the cellular composition of normal marrow or alter the marrow response to low oxygen tension (19).

Betha (20) have reported striking morphological changes in the vascular system of the bone marrow in cases of polycythemia vera. These include thickening and sclerosis of the vascular walls and the occurrence of thromboses. We have examined the marrows of our cobalt polycythemic animals for these vascular changes, using the same technique described by Reznikoff *et al.* No such changes were found and in fact there was no evidence of any morphological changes in the vessels. Dorrance and his collaborators (4) have also reported recent pathologic studies in which no vascular changes were noted.

DISCUSSION. In the foregoing sections, we have been unable to confirm the thesis that cobalt acts by impairing the respiratory activity of the bone marrow and have shown that its effects are independent of the peripheral innervation of the marrow and do not involve morphological changes in the marrow vessels. How it acts is certainly not elucidated, but in the spirit of stimulating further investigation, two possibilities might be entertained. 1. It could act on the liver, possibly enhancing the formation there of metabolic precursors requisite for red cell production. That choline, its antagonist, acts there is well known. 2. The above hepatic mechanism might be set off by cobalt acting on a central neural mechanism. Incidentally, a hypothetical case may be made out for oxygen lack acting by way of a central neural mechanism also, but further speculation along these lines is obviously unwarranted.

SUMMARY AND CONCLUSIONS

1. The observation of Barron and Barron that the respiration of bone marrow and reticulocytes *in vitro* is impaired by small amounts of cobalt cannot be confirmed.

2. The respiration and glycolysis of the marrow of cobalt-polycythemic animals is slightly lower than that which would be predicted from previous studies, but the difference is so slight as to be of very doubtful significance.

3. The *in vitro* respiration of both normal and polycythemic marrow is not altered by substituting polycythemic for normal serum.

4. The erythroid hyperplasia of bone marrow in cobalt-polycythemic animals is independent of whether or not the marrow has an intact peripheral innervation.

5. No morphological changes were observed in the blood vessels of marrow from cobalt-polycythemic animals.

6. In our opinion, the mechanism of the action of cobalt in inducing polycythemia is unexplained; several possibilities are suggested for further study.

REFERENCES

- (1) BARRON, A. G. AND E. S. G. BARRON. Proc. Soc. Exper. Biol. and Med. **35**: 407, 1936.
- (2) MASCHERPA, P. Haematologia **10**: 361, 1929.
- (3) KLEINBERG, W., A. S. GORDON AND H. A. CHARIPPER. Proc. Soc. Exper. Biol. and Med. **42**: 119, 1939.
- (4) DORRANCE, S. S., G. W. THORN, M. CLINTON, JR., H. W. EDMONDS AND S. FARBER. This Journal **139**: 399, 1943.
- (5) WALTNER, K. AND K. WALTNER. Klin. Wchnschr. **8**: 313, 1929.

- (6) ORTEN, J. M. This Journal 114: 414, 1936.
- (7) WARREN, C. O. This Journal 131: 176, 1940.
- (8) WARREN, C. O. This Journal 128: 455, 1939.
- (9) STARE, F. J. AND C. A. ELVEHJEM. J. Biol. Chem. 99: 473, 1933.
- (10) COPP, D. H. AND D. M. GREENBERG. Proc. Nat. Acad. Sci. 27: 153, 1941.
- (11) MULLER, O. H. The polarographic method of analysis. J. Chem. Education, Easton, Pa., 1941.
- (12) DAVIS, J. E. This Journal 127: 322, 1939.
- (13) DAVIS, J. E. This Journal 129: 140, 1940.
- (14) DAVIS, J. E. J. Pharmacol. and Exper. Therap. 70: 408, 1940.
- (15) DAVIS, J. E. This Journal 134: 219, 1941.
- (16) DAVIS, J. E. This Journal 137: 94, 1942.
- (17) BURNS, B. I. J. Comp. Neurol. 61: 191, 1935.
- (18) KLEINBERG, W. This Journal 108: 545, 1934.
- (19) WARREN, C. O. This Journal 135: 249, 1941.
- (20) REZNIKOFF, P., N. C. FOOT AND J. M. BETHEA. Am. J. Med. Sci. 189: 753, 1935.

THE BLOOD PICTURE OF IRON AND COPPER DEFICIENCY ANEMIAS IN THE RABBIT

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A previous paper (Smith and Medlicott, 1944) described the changes which occurred in the blood picture of rats that were fed diets deficient in iron and/or copper. The present paper extends these studies to the rabbit.

The techniques used were described in detail in the above mentioned paper, and only a summary will be given here. The rabbits were secured from the laboratory colony of Dutch rabbits. When the young rabbits were approximately two weeks of age, all feed was removed from the breeding cages, and thereafter the young were given access only to milk enriched with 10 per cent of whole milk powder (Klim) which was later supplemented with either iron or copper. The nursing females were removed for approximately a four-hour period daily to another cage and permitted to consume the stock diet of G. L. F. calf pellets and a mixed clover hay after which they were returned to their young for the remainder of the day. The young were weaned at four weeks of age and placed in individual, galvanized wire cages and continued on the milk diet, which was fed ad lib.

Blood samples were taken from an ear vein at weekly intervals until the hemoglobin dropped to about 4 grams per 100 ml. of blood (approximately four to six weeks after weaning) at which time 1 ml. of blood was taken by heart puncture, mixed with heparin to prevent coagulation and subjected to the following determinations: hemoglobin, red cell count, red cell volume and reticulocyte count. All of these determinations were performed in duplicate. In addition, blood smears were stained with Wright's solution for microscopic examination. In taking blood samples from the ear veins, it was found very helpful first to rub the ear with a cloth moistened with ether. This brought about a dilatation of the veins and a good flow of blood when the veins were punctured with a lancet.

Rabbits hereafter referred to as milk anemic rabbits were those which subsisted on the milk diet only. Rabbits which are referred to as iron-fed were those which developed a severe anemia on the milk diet and were then supplemented with 2 mgm. of pure iron per rabbit per day. The source of this iron was iron carbonyl¹. The rabbits designated as copper-fed were those which developed a severe anemia on the milk diet and were then fed 0.2 mgm. of copper per rabbit per day. The source of this copper was electrolytic copper. Blood samples were taken by heart puncture after two to four weeks following iron or copper supplementation and subjected to the various determinations previously mentioned. The rabbits called normal were litter mates of the anemic rabbits which

¹ General Aniline Works, Grasselli, New Jersey.

received our stock diet. Blood samples were taken from these rabbits when they were ten to eleven weeks of age.

RESULTS. Blood analyses were obtained on 32 milk-anemic, 7 iron-fed and 8 copper-fed rabbits. It was planned to have larger numbers of observations in the latter two groups, but a high rate of mortality, particularly as the hemoglobin fell to a low level, prevented this.

The hemoglobin response of milk anemic rabbits when fed iron or copper is given in table 1.

TABLE 1

The hemoglobin response of milk anemic rabbits when fed iron or copper

RABBIT	IRON (2 MCM./DAY) HEMOGLOBIN (GRAMS/100 ML. BLOOD)		RABBIT	COPPER (0.2 MCM./DAY) HEMOGLOBIN (GRAMS/100 ML. BLOOD)		
	Initial	2 wks.		Initial	1 wk.	2 wks.
1	4.0	3.8	8	3.8	3.0	1.9
2	3.7	2.7	9	4.0	4.4	3.3
3	4.4	7.5*	10	4.1	3.3	3.0
4	2.8	1.8	11	4.0	4.2	3.8
5	5.1	3.1	12	3.4	4.5	4.2
6	4.1	2.9	13	5.0	4.5	3.7
7	3.0	1.3	14	5.0	4.7	4.1
			15	4.2	4.5	3.5
Ave.....	3.87	3.30		4.19	4.14	3.44

* Hemoglobin fell to 4.4 grams in five weeks.

TABLE 2

A summary of the blood picture of normal, milk-anemic, iron-fed and copper-fed rabbits.

TREATMENT	NO.	HEMOGLOBIN	R.B.C.	HEMA- TOCRIT	M.C.V.	M.C.Hb. CONC.	M.C. Hb	RET.
		gms./100 ml	millions/cmm.	%	μ^3	%	rr	%
Normal.....	13	14.02 \pm 0.297	6.526 \pm 0.1389	42.3 \pm 0.86	65.0 \pm 1.02	33.1 \pm 0.52	21.5 \pm 0.20	1.2 \pm 0.20
Milk anemic..	32	3.84 \pm 0.123	2.482 \pm 0.1407	13.6 \pm 0.53	56.7 \pm 1.50	28.6 \pm 0.55	16.3 \pm 0.62	5.4 \pm 0.91
Fe-fed.....	7	2.52 \pm 0.411	1.402 \pm 0.1664	8.3 \pm 1.19	58.3 \pm 4.22	30.0 \pm 1.20	17.6 \pm 1.73	2.3 \pm 0.29
Cu-fed.....	8	3.07 \pm 0.246	2.676 \pm 0.3015	11.8 \pm 0.94	47.3 \pm 2.39	26.1 \pm 0.44	12.0 \pm 0.80	10.1 \pm 2.35

The absence of a hemoglobin response following either iron or copper supplementation is interpreted as meaning that the iron used was free of copper and that the copper used was free of iron. In a few cases where 2 mgm. of iron and 0.2 mgm. of copper were fed simultaneously, rapid hemoglobin regeneration occurred.

A summary of the blood picture of normal and anemic rabbits is given in table 2. This includes the mean hemoglobin values, red blood cell counts (R.B.C.), the centrifuge hematocrits, and the indices calculated from the foregoing values—mean red cell volume (M.C.V.), mean red cell hemoglobin concentration (M.C. Hb. Conc.), and mean red cell hemoglobin content (M.C. Hb.). Also included are the mean reticulocyte counts. The significance of mean differences was determined by Student's *t* test. A *P* value of 0.01 is considered highly significant,

and a P value of 0.05 is considered significant. The mean cell volume, mean cell hemoglobin concentration, and mean cell hemoglobin content of the blood of the milk anemic rabbits are all less than in normal rabbits. These differences are highly significant statistically. Thus, in the rabbit, milk anemia is microcytic and hypochromic. The mean cell volume, mean cell hemoglobin concentration, and mean cell hemoglobin of iron-fed rabbits did not differ significantly from those of the milk anemics. In the case of copper-fed rabbits, the microcytosis and hypochromasia were more severe than in the case of the milk anemics as shown by a significant decrease in mean cell volume and a highly significant decrease in the mean cell hemoglobin concentration. The anemia in all three cases was microcytic and hypochromic.

The reticulocytes showed a highly significant increase over normal in the case of the milk anemics and the copper-fed rabbits. The copper-fed rabbits showed a highly significant increase over the milk anemics.

In the rat, it was shown that copper stimulated erythropoiesis in milk anemic animals (Smith and Medlicott). The highly significant increase in the reticulocyte counts in the copper-fed rabbits as compared to those of the milk anemic rabbits indicates that this observation also holds true for the rabbit. Some additional support for this idea is found in the red cell counts of the two groups. In spite of the fact that the mean hemoglobin value of the copper-fed group is lower than that of the milk anemic group, the mean red cell count of the copper-fed animals is higher than that of the milk anemic rabbits, although this difference is not statistically significant.

A microscopic examination of the blood smears confirmed the microcytosis and hypochromasia, which was particularly evident in the smears of the copper-fed rabbits. In addition, there was a mild poikilocytosis. Basophilia was not pronounced in any of the smears.

DISCUSSION. The data in this experiment show that in the rabbit a deficiency of iron or copper or both in the diet of rabbits leads to a hypochromic and microcytic anemia. A similar situation prevails in the rat (Smith and Medlicott, 1944; Foster, 1931; Fitz-Hugh *et al.*, 1933). In man, a deficiency of iron is known to produce a microcytic and hypochromic anemia. The only exception so far as the authors are aware is the pig where, according to Hamilton *et al.* (1933), a milk anemia results in a hypochromic and normocytic anemia.

CONCLUSIONS

A deficiency of iron or copper or both in the diet produces a microcytic and hypochromic anemia in the rabbit.

REFERENCES

- FITZ-HUGH, T., G. M. ROBSON AND D. L. DRABKIN. *J. Biol. Chem.* 103: 617, 1933.
FOSTER, P. C. *J. Nutrition* 4: 517, 1931.
HAMILTON, T. S., G. E. HUNT AND W. E. CARROLL. *J. Agric. Res.* 47: 543, 1933.
SMITH, S. E. AND M. MEDLICOTT. *This Journal* 141: 354, 1944.

THE INFLUENCE OF GLUCOSE RENAL TUBULAR REABSORPTION AND P-AMINOHIPPURIC ACID TUBULAR EXCRETION ON THE SIMULTANEOUS CLEARANCE OF ASCORBIC ACID

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In a previous report it was demonstrated that the infusion of hypertonic potassium and sodium chloride markedly reduced the simultaneous renal tubular reabsorption of ascorbic acid (1). Since there occurred no associated decrease in glomerular filtration rate or effective renal plasma flow, and since osmotic diuresis did not influence the rate of ascorbic acid reabsorption, it was inferred that these salts blocked the tubular transfer of ascorbic acid in some aspecific manner.

The purpose of the present report is to analyze further the interrelationship of certain tubular processes by means of studying the effects of the renal clearance of other substances involving tubular mechanisms on simultaneous ascorbic acid reabsorption. One of these, glucose, is reabsorbed by the tubules (2), and the other, p-aminohippuric acid, is excreted by the tubules (3). Related tubular processes should then be revealed by the interference on the part of these substances with the reabsorption of ascorbic acid.

In this study particular attention has been directed to the following relationships: *a*, the effect of saturation of the p-aminohippuric acid excretory mechanism on reabsorption of ascorbic acid; *b*, the effect of saturation of the glucose reabsorptive mechanism on simultaneous ascorbic acid reabsorption; *c*, the effect of saturation of both mechanisms on ascorbic acid reabsorption; and *d*, the effect of saturation of the p-aminohippuric excretory mechanism on glucose reabsorption.

EXPERIMENTAL PROCEDURE. Six trained female dogs ranging in weight from 10 to 19 kgm. were used in various phases of this study. They were kept on a mixed diet. Clearance experiments were conducted by maintaining desired plasma levels of the substances to be cleared by constant intravenous infusion. To allow suitable time for equilibration, collection of the urine in approximately ten minute intervals was begun about thirty minutes after the initial priming dose, the bladder being rinsed at the end of each collection period. Adequate urine flows were insured by hydrating the dogs with 40 cc. of water per kgm. about one hour before the beginning of each clearance experiment.

The general plan of each clearance experiment was to make three observations of the normal maximal rate of ascorbic acid reabsorption, after which priming doses of p-aminohippuric acid or glucose were followed by continuous infusion of the respective substances and continued for three more ten-minute collection periods. In additional experiments, both glucose and ascorbic acid were given

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during the control periods, followed by p-aminohippuric priming and infusion. In all cases the rates of infusion of the substances were more than adequate to saturate their respective tubular mechanisms.

The tubular reabsorption of ascorbic acid and glucose was calculated as the difference between the amount filtered (glomerular filtration rate in cubic centimeters per minute \times plasma concentration) and the amount concurrently excreted (urinary concentration \times rate of urine flow). The clearance of simultaneously infused creatinine was taken as the measure of glomerular filtration rate. Creatinine content of CdSO₄ plasma filtrates (4) and diluted urines was measured in

TABLE 1

A typical experiment showing the effect of p-aminohippuric acid tubular excretion on the simultaneous reabsorption of ascorbic acid

During the control periods p-aminohippuric was excreted at a rate approximately 25 per cent of the maximal

	ASCORBIC ACID						D-AMINOHIPPURIC	
	Plasma conc.	Glom. filt. rate	Filtered	Excreted	Reabsorbed	Clearance ratio: ascorbic creatinine	Excreted	Amt. excreted Tm*
	mgm./%	cc./min.	mgm./min.	mgm./min.	mgm./min.		mgm./min.	
Control	4.00	40.0	1.600	1.310	0.290	0.816	3.18	0.246
	4.20	41.3	1.735	1.485	0.250	0.855	3.25	0.253
	4.40	42.7	1.880	1.640	0.240	0.872	3.36	0.262
	4.20	41.3	1.740	1.478	0.260	0.848	3.26	0.254
During p-amino-hippuric Tm: Time								
12 min.....	4.58	43.0	1.970	1.970	0.000	1.000	13.3	1.04
22 min.....	4.70	40.0	1.880	1.830	0.050	0.975	11.9	0.93
32 min.....	4.88	40.5	1.975	1.930	0.045	0.975	13.2	1.03
	4.72	41.3	1.941	1.910	0.031	0.983	12.8	

* The average of the three periods during which p-aminohippuric acid was infused at elevated plasma levels is taken as Tm.

the photoelectric colorimeter by the alkaline-picrate method of Folin and Wu (5). Plasma and urinary ascorbic acid concentration was determined by the dichloro-indophenol method in the photoelectric colorimeter with modifications given in a previous report (6), after demonstration that simultaneously infused substances did not interfere with the test. Glucose was measured by Shannon's modification of the Folin method (7). Determination of p-aminohippuric acid was made after the method developed by Smith (8). All analyses were made in duplicate.

RESULTS. A. *The effect of saturation of the p-aminohippuric acid excretory mechanism on the reabsorption of ascorbic acid.* When the tubular excretion of

p-aminohippuric acid was elevated from considerably below maximal to the maximal rate (T_m), the reabsorption of ascorbic acid was immediately blocked, but soon progressively began to recover (tables 1 and 2, fig. 1). In only one experiment, however, was there indication that reabsorption had returned to normal within fifty minutes after tubular saturation by p-aminohippuric acid had been effected. While these changes in ascorbic acid reabsorption were occurring, the

TABLE 2

Summary of the effects of low and maximal rates of p-aminohippuric acid tubular excretion on the simultaneous reabsorption of ascorbic acid

DOG	ASCORBIC ACID							p-AMINOHIPPURIC	
	Plasma conc.	Glom. filt. rate	Filtered	Excreted	Reab-sorbed	Amt. reabs. T_m^*	Clearance ratio ascorbic creatinine	Excreted	Amt. excr. T_m^\dagger
	mgm./%	cc./min.	mgm./min.	mgm./min.	mgm./min.			mgm./min.	
D	3.47	69.1	2.390	1.720	0.670		0.720	5.00	0.290
	3.81	73.0	2.782	2.635	0.147	0.220	0.950	17.40	
	3.16	83.3	2.623	1.893	0.730		0.720	3.10	0.210
	3.70	75.0	2.773	2.417	0.357	0.490	0.870	14.90	
M	3.88	64.0	2.487	1.850	0.638		0.750	2.90	0.180
	3.10	58.4	1.815	1.540	0.270	0.420	0.850	16.40	
	2.71	70.7	1.917	1.307	0.610		0.680	0.00	
	2.88	65.0	1.877	1.583	0.294	0.480	0.850	16.30	
P	3.17	57.5	1.817	1.350	0.468		0.730	2.50	0.100
	3.50	62.4	2.180	2.080	0.100	0.210	0.950	25.00	
H	4.20	41.3	1.740	1.478	0.260		0.850	3.30	0.260
	4.72	41.3	1.941	1.910	0.031	0.122	0.980	12.80	

* Ascorbic acid T_m value represents the average of the three control period determinations done at load levels adequate to insure saturation of the reabsorptive mechanism.

† p-aminohippuric acid excretory T_m represents the average of the three periods during which the excretion by the tubules was maximum at plasma levels which were adequate to insure saturation of the mechanism.

Each figure in the above table represents the average of three consecutive ten-minute collection periods.

maximal rate of excretion of p-aminohippuric acid remained essentially constant.

When the rate of transfer of p-aminohippuric acid was below the tubular maximum, the concurrent reabsorption of ascorbic acid appeared to be within the normal range. This is evident in table 3, where sufficient control data are available for three of the dogs used in the present study to permit comparison. Hence the effects on the ascorbic acid mechanism occurred only when the rate of transfer of p-aminohippuric acid was maximal.

It is clear from figure 1 that the infusion of p-aminohippuric acid has no significant effect on the rate of glomerular filtration. From this it must be concluded

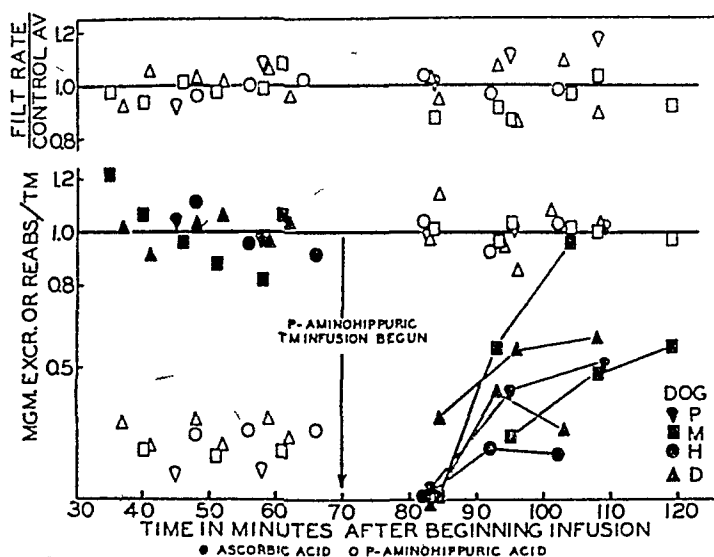


Fig. 1. The effect of the tubular excretion of p-aminohippuric acid on the simultaneous reabsorption of ascorbic acid and on the glomerular filtration rate is here shown. During the control periods (left) p-aminohippuric acid was infused so that the excretory capacity was only about 20 per cent saturated. At an average time of 70 minutes from the beginning of the clearance, infusion of p-aminohippuric acid was elevated to a rate more than adequate to saturate the excretory mechanism.

Each point represents the average value of one ten-minute collection period, expressed as a ratio to the average of three consecutive control periods. The p-aminohippuric excretion during the control periods is taken as a ratio to the average of three consecutive T_m determinations.

TABLE 3

Comparison of ascorbic acid reabsorption without and with simultaneous excretion of p-aminohippuric acid at rates considerably below maximal

DOG	CONTROL						WITH p-AMINOHIPPURIC INF.:			
	Ascorbic acid						Ascorbic acid			p-amino-hippuric excretion Tm†
	No. of expts.	Range (days)*	Load (mgm./min.)		Tm (mgm./min.)		Load (mgm./min.)	Tm (mgm./min.)	Dev. from normal (%)	
			Av.	Range	Av.	Range				
D	9	56	1.547	1.142-2.033	0.603	0.45-0.77	2.633	0.73	+17	0.210
							2.390	0.67	+11	0.290
M	9	34	1.880	1.409-2.555	0.555	0.40-0.71	2.487	0.64	+15	0.180
P	5	45	1.954	1.133-3.273	0.534	0.37-0.60	1.817	0.47	-12	0.100

* This represents the range in days from the experimental observation in which the control observations were made.

† The T_m values for p-aminohippuric excretion appear in table 2.

that the results noted were not caused by glomerular shut-down, but that this substance exerted its effect by blocking ascorbic acid tubular reabsorption.

B. *The effect of saturation of the glucose reabsorptive mechanism on simultaneous ascorbic acid reabsorption.* The immediate effect of saturation of the glucose reabsorptive mechanism was to block the simultaneous reabsorption of ascorbic acid (fig. 2A and table 4). As time progressed, however, ascorbic acid reabsorption began again and continued to increase, but did not return to a maximal rate during sixty minutes of observation. Similar effects were noted in the data of figure 2B and table 5 where during the control periods both glucose and ascorbic acid were infused in several dogs for time intervals equivalent to the above experiments. Accordingly, after an average of 51 minutes of glucose infusion the

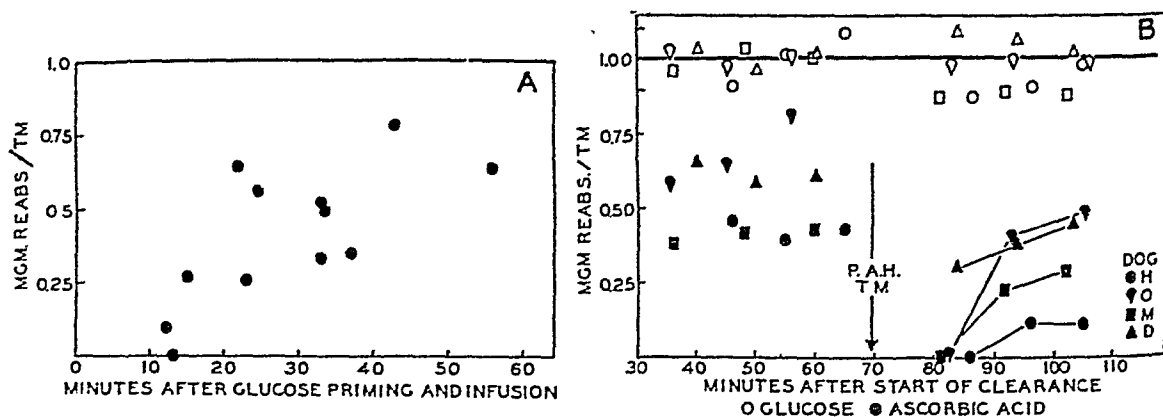


Fig. 2. A. This shows the effect of saturation of the glucose reabsorptive mechanism on the simultaneous ascorbic acid reabsorption. Each point represents the ratio of the amount reabsorbed during a single collection period to the average reabsorption of three control periods done at load levels adequate to saturate the ascorbic mechanism. All values were obtained in four experiments with the same dog.

B. The effect of p-aminohippuric acid excretion at a maximal rate on the simultaneous reabsorption of glucose and ascorbic acid. The experimental periods for glucose (open symbols) are expressed as ratios to the average of three consecutive control periods done before p-aminohippuric Tm was begun.

The ascorbic acid values (solid symbols) are all expressed as ratios to the normal ascorbic acid Tm in these dogs when no glucose was infused. The normal Tm values in milligrams per minute were: dog H: 0.58; dog O: 0.37; dog M: 0.60; dog D: 0.65. These values represent the averages of from two to five clearance experiments done within 19 days of the above experiments.

rate of ascorbic acid reabsorption was still only 53 per cent of the normal maximal value.

During this time the glucose Tm remained substantially constant (within an average deviation of ± 4 per cent of the mean); nor did the glomerular filtration rate vary significantly in all the experiments in which glucose was given (within an average deviation of ± 6 per cent of the mean). Thus here again the effects did not result from glomerular closure, but rather it must be concluded that while glucose is passing through the tubular cells at a maximal rate ascorbic acid reabsorption at least temporarily ceases and does not return to normal during the time of observation.

C. *The combined effects of maximal rates of transfer of glucose and p-amino-*

hippuric acid on that of ascorbic acid. During the control periods of four experiments which bear on this point, both glucose and ascorbic acid were infused at rates adequate to saturate their respective mechanisms. As has been stated, under these conditions one may expect the ascorbic acid T_m to be depressed while the glucose T_m remains normal. When the tubular excretory mechanism was then saturated with p-aminohippuric acid, the reabsorption of ascorbic acid was completely blocked (fig. 2B and table 5). It again typically began to recover while both glucose reabsorption and p-aminohippuric excretion continued at a constant rate, but did not return to its original value in the time observed. These experiments further support the argument that ascorbic acid reabsorption is linked in some way with both tubular excretion and reabsorption of other sub-

TABLE 4

A typical experiment showing the effect of maximal tubular glucose reabsorption on the simultaneous reabsorption of ascorbic acid

	ASCORBIC ACID						GLUCOSE		
	Plasma conc.	Glom. filt. rate	Filtered	Excreted	Reabsorbed	Clearance ratio ascorbic creatinine	Filtered	Excreted	Reabsorbed
	mgm./%	cc./min.	mgm./min.	mgm./min.	mgm./min.		mgm./min.	mgm./min.	mgm./min.
Control	3.00	94.2	2.820	2.042	0.778	0.740			
	3.00	99.0	2.970	2.280	0.690	0.760			
	3.07	93.0	2.850	2.220	0.630	0.775			
	3.02	95.4	2.880	2.181	0.699	0.758			
During glucose T_m : Time									
12 min.....	3.18	89.5	2.846	2.780	0.066	0.980	443.0	199.0	244.0
22 min.....	2.96	92.7	2.740	2.290	0.450	0.835	510.0	253.0	257.0
33 min.....	2.77	89.0	2.460	2.100	0.360	0.854	548.0	279.0	269.0
	2.97	90.4	2.682	2.390	0.292	0.890	500.0	243.0	257.0

stances, for they demonstrate that glucose and p-aminohippuric acid may simultaneously depress ascorbic acid reabsorption.

Whenever any of the above mentioned procedures reduce ascorbic acid tubular reabsorption, the clearance of the substance is of course elevated. Thus, with reabsorption fairly completely blocked, the ascorbic acid/creatinine clearance ratio approaches unity. Reference to the tables will exemplify this trend.

D. *The effect of saturation of the p-aminohippuric excretory mechanism on simultaneous glucose reabsorption.* While p-aminohippuric acid was being maximally excreted by the tubules, there was no effect on the glucose T_m , even while the ascorbic acid T_m was reduced (fig. 2B and table 5). The present experiments have not been designed to examine whether the reciprocal effect is true, i. e.,

whether glucose tubular saturation influences the maximal rate of excretion by the tubules. Data from other workers indicate that such is not the case either in the dog (9) or in man (10).

TABLE 5

The effect of a maximal rate of tubular excretion of p-aminohippuric acid on the simultaneous reabsorption of glucose and ascorbic acid

DOG		p-AMINO- HIPPURIC EX- CRETED	GLOM. FILT. RATE	GLUCOSE					ASCORBIC ACID				
				Plasma	Fil- tered	Ex- creted	Reab- sorbed	Amt. reabs. Tm†	Plas- ma	Fil- tered	Ex- creted	Reab- sorbed	Clear- ance ratio ascorbic creati- nine
		mgm./ min.	cc./ min.	mgm./ %	mgm./ min.	mgm./ min.	mgm./ min.		mgm./ %	mgm./ min.	mgm./ min.	mgm./ min.	
H	Control*...		47.3	943.0	446.0	292.0	154.0		3.60	1.700	1.452	0.248	0.855
	During....	10.00	45.7	980.0	448.0	314.0	134.0	0.87	6.10	2.788	2.782	0.006	1.000
	p.a.h.	9.60	41.0	1000.0	410.0	271.0	139.0	0.90	5.83	2.390	2.325	0.065	0.970
	Tm.....	11.10	39.4	1010.0	398.0	247.0	151.0	0.98	5.60	2.225	2.163	0.062	0.983
O	Control . .		32.7	782.0	255.0	139.0	116.0		3.93	1.285	1.034	0.251	0.804
	During....	8.30	33.0	855.0	282.0	169.0	113.0	0.97	5.18	1.706	1.700	0.006	1.000
	p.a.h.....	6.40	35.5	815.0	289.0	173.0	116.0	1.00	4.85	1.722	1.575	0.147	0.913
	Tm.....	7.80	39.0	775.0	302.0	188.0	114.0	0.98	4.50	1.755	1.580	0.175	0.902
M	Control ...		58.5	536.0	313.0	101.0	212.0		2.65	1.551	1.305	0.246	0.843
	During....	13.70	55.7	428.0	238.0	54.0	184.0	0.87	2.35	1.309	1.305	0.004	1.000
	p.a.h. . . .	13.20	60.4	413.5	249.5	61.0	188.5	0.89	2.40	1.450	1.315	0.135	0.910
	Tm.....	14.30	59.0	404.5	238.5	52.0	186.5	0.88	2.47	1.455	1.280	0.175	0.877
D	Control ...		83.5	703.0	587.0	289.0	298.0		2.81	2.347	1.950	0.397	0.830
	During ...	12.40	75.5	776.0	586.0	262.0	324.0	1.09	3.71	2.800	2.605	0.195	0.932
	p.a.h.	14.10	84.0	726.0	609.0	293.0	316.0	1.06	3.88	3.260	3.015	0.245	0.925
	Tm.....	12.50	79.3	707.0	562.0	259.0	303.0	1.02	4.05	3.210	2.920	0.290	0.910

* The control figures represent the average of three consecutive ten-minute collections.

† The Tm for glucose represents the average of the three control periods at load levels adequate for saturation of the glucose reabsorptive mechanism.

The ascorbic acid Tm for the above dogs when no glucose was infused was as follows: dog H: 0.580; dog O: 0.370; dog M: 0.600; dog D: 0.647. These represent the averages of two to five clearance experiments done within 19 days of the above clearances.

DISCUSSION. In searching for the possible mode of interference of these substances with ascorbic acid reabsorption, the effect of the high osmotic activity of glucose and p-aminohippuric acid in the proximal tubules must be considered, in view of the possibility that interference with normal water reabsorption may influence the reabsorption of ascorbic acid. Against this possibility, however, the following arguments may be advanced: *a*, water diuresis over a wide range of

urine flows has no significant influence on ascorbic acid T_m ; *b*, no correlation was found between the amounts of glucose and p-aminohippuric excreted and ascorbic acid reabsorption; *c*, when an osmotic diuretic that is itself not reabsorbed by the tubules is given (mannitol), there is no significant effect on ascorbic acid reabsorption (1). Hence some process of competition between the tubular transport mechanism seems necessary to explain the above results.

Pitts (11), largely on the evidence that glycine, alanine, and glutamic acid completely or partially block the simultaneous reabsorption of creatine, has argued that the amino acids are reabsorbed by a common mechanism, and that a single cellular element is common to this mechanism. Shannon had earlier made the same conclusion with regard to the behaviour of glucose and xylose (12). Applying similar reasoning, the demonstration that both glucose and chlorides, reabsorbed by the tubules, and p-aminohippuric acid, excreted by the tubules, reversibly block ascorbic acid reabsorption must lead to the conclusion that the tubular transfer mechanisms of these substances possess some common link. But if it is concluded that a common cellular element is involved, one is forced to the seemingly irresolvable paradox that reabsorption and excretion can proceed simultaneously while using the same transport substance.

Shannon, in applying the mass law to tubular transport (12), admits that the reversible combination of a substance with the "cellular element" proceeds at the expense of energy produced by reactions which escape analysis by reason of their probable complexity. While the present evidence does not preclude the possibility that individual (or closely related) substances may have specific tubular transport mechanisms, it does suggest that other interrelationships of tubular processes exist, possibly in the energy producing systems, for this would explain competition of a non-specific nature such as observed in the present study.

Other evidence exists that there must be common links between renal tubular processes of excretion and reabsorption. Phlorizin inhibits certain processes of tubular reabsorption (glucose, xylose (13) and ascorbic acid (14)) as well as the tubular excretion of creatinine (13), diodrast, and phenol red (15,16). Similarly, Eiler, Althausen and Stockholm have demonstrated that thyroxine increases both the rate of transfer of glucose and diodrast in the same animal (9) and have stressed the rôle of phosphorylation as a transmitter of oxidative energy. They propose that thyroid hormone influences the activity or availability of some factor involved in the transfer of phosphate bond energy, and suggest that adenosine-triphosphatase may be such a factor.

However, certain difficulties are presented by the present evidence in applying the concept that the non-specific inter-relationship observed between ascorbic acid, glucose, and p-aminohippuric acid is at the energy level of tubular transport. In the first place, there appears to be no limit to the total available tubular energy, for both glucose and p-aminohippuric acid transfer proceeds simultaneously in a normal manner. Furthermore, the energy requirements for ascorbic acid transfer are apt to be low, on the basis of the absolute T_m , when compared to such substances as glucose or p-aminohippuric acid. Thus, if the interpretation is permitted that competition exists at the energy level, one must conclude that the ascorbic acid transfer mechanism has a specific orientation or affinity for the

energy system which can be readily modified by a maximal rate of transfer of other substances which require energy.

Favoring such an interpretation is the observation that after the reabsorption of ascorbic acid has been blocked for a time, it begins again and gradually increases in rate. The flexibility of this relationship would seem to be characteristic of the energy producing system, in that it adjusts itself to the existing tubular transfer processes, but does so slowly for the demands of ascorbic acid reabsorption.

SUMMARY AND CONCLUSIONS

1. When p-aminohippuric acid is excreted at a maximal rate by the renal tubules, the simultaneous reabsorption of ascorbic acid is temporarily blocked. It gradually begins again but does not recover its original rate during fifty minutes of observation.

2. Similarly, when glucose is being reabsorbed at a maximal rate, ascorbic acid reabsorption is at first completely blocked, and in the same manner undergoes partial recovery.

3. Although their respective tubular mechanisms do not mutually interfere, glucose and p-aminohippuric acid when simultaneously infused depress ascorbic acid reabsorption to a greater degree than does each substance singly. From this it is concluded that the observed interference could not have resulted from competition for a specific transport mechanism.

4. Because of the non-specific nature of the competition, the view is favored that the observed interrelationship is in the system which supplies energy for tubular transfer.

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REFERENCES

- (1) SELKURT, E. E. AND C. R. HOUCK. *This Journal* **141**: 423, 1944.
- (2) SHANNON, J. A. AND S. FISHER. *This Journal* **122**: 765, 1938.
- (3) FINKELSTEIN, N., L. M. ALIMINOSA AND H. W. SMITH. *This Journal* **133**: P276, 1941.
- (4) FUJITA, A. AND D. IWATAKE. *Biochem. Ztschr.* **242**: 43, 1931.
- (5) FOLIN, O. AND H. WU. *J. Biol. Chem.* **38**: 81, 1919.
- (6) SELKURT, E. E., L. J. TALBOT AND C. R. HOUCK. *This Journal* **140**: 260, 1943.
- (7) SHANNON, J. A., S. FARBER AND L. TROAST. *This Journal* **133**: 752, 1941.
- (8) SMITH, H. W. Unpublished.
- (9) EILER, J. J., T. L. ALTHAUSEN AND M. STOCKHOLM. *This Journal* **140**: 699, 1944.
- (10) SMITH, H. W., W. GOLDRING, H. CHASIS, H. A. RANGES AND S. E. BRADLEY. *J. of Mt Sinai Hospital* **10**: 59, 1943.
- (11) PITTS, R. F. *This Journal* **140**: 535, 1944.
- (12) SHANNON, J. A. *This Journal* **122**: 775, 1938.
- (13) SMITH, H. W. *The physiology of the kidney*. Oxford Press, 1937.
- (14) PIANTONI, C. *Rev. Soc. Argentina biol.* **16**: 175, 1940.
- (15) WHITE, H. L. *This Journal* **130**: 582, 1940.
- (16) CHASIS, H., H. A. RANGES, W. GOLDRING AND H. W. SMITH. *J. Clin. Investigation* **17**: 683, 1938.

THE EFFECT OF ADRENAL CORTICAL EXTRACT ON THE RESISTANCE OF NON-ADRENALECTOMIZED RATS TO PEPTONE SHOCK

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In an earlier study from this laboratory (1) it was shown that extracts and certain steroids from the adrenal cortex were without effect upon the resistance of non-adrenalectomized rats to shock caused by the ligation of the two hind limbs. These studies on shock have been extended by using a toxic substance as the damaging agent.

Shock was induced in non-adrenalectomized rats by the intraperitoneal injection of peptone. The injection of large doses of adrenal cortical extracts increased the incidence of survival over that of untreated animals. The administration of 11-desoxycorticosterone acetate and of small doses of adrenal cortical extracts were ineffective.

METHODS. Normal male rats of the Sprague-Dawley strain, weighing 185 to 195 grams, were used. The diet was Purina dog chow. The animals were not fasted prior to the beginning of the experiment but during the experiment food and water were withheld. A solution of peptone (Bacto-Peptone, Difco) in water containing 50 grams per 100 cc. was used. Shock was induced by the intraperitoneal injection of 3.5 cc. of peptone solution in each rat.

Adrenal cortical extracts from beef and from hog adrenal glands were made up in sesame oil solution so that each dose was contained in 0.5 cc. All traces of epinephrine had been removed from these preparations. A control solution was prepared by dissolving physiologically inert material from adrenal extracts in sesame oil so that it could not be distinguished from the active extract on the basis of appearance. The compound, 11-desoxycorticosterone acetate was dissolved in sesame oil and as a control sesame oil only was used.

All test solutions were submitted to the experimenter as "unknowns". The selection and matching of the rats with the test substances was by the following procedure. The rats were first matched into pairs on the basis of body-weight. All of the rats were then injected with peptone. A card was drawn at random from a thoroughly shuffled pack and the matching of the rats of each pair with the test solutions was made according to the designations on the card.

EXPERIMENTS AND RESULTS. The test solutions were administered immediately following the injection of peptone and again seven, twenty-four and thirty-one hours later. The incidence of survival at seven, twenty-four, thirty-one and forty-eight hours following the injection of peptone was the criterion used to judge the efficacy of the test substances.

The data on dosage and incidence of survival of rats treated with cortical ex-

tracts are summarized in table 1. The data on rats treated with 11-desoxycorticosterone acetate are summarized in table 2.

The administration of cortical extract in the amount equivalent to 25 grams of beef adrenal glands per dose was ineffective. When larger amounts of extracts of beef adrenal glands were given the incidence of survival was uniformly greater

TABLE 1

The effect of adrenal cortex extract on the number of non-adrenalectomized male rats (185 to 195 grams) surviving the intraperitoneal injection of 3.5 cc. of peptone

SOLUTION	GLAND (BEEF) EQUIVALENT	NUMBER TESTED	HOURS FOLLOWING INJECTION OF PEPTONE			
			7	24	31	48
285	25 grams/dose	50	42	15	10	9
285A	control	50	36	18	13	11
285	50 grams/dose	50	43	26	16	14
285A	control	50	44	13	10	7
284B	25 grams/dose	50	48	25	10	8
284C	control	50	45	21	14	12
287A	50 grams/dose	50	40	23	17	16
287B	control	50	39	19	10	9
99C	50 grams/dose	50	44	22	16	14
99F	control	50	39	19	14	12
99D	50 grams/dose	50	26	16	15	15
99G	control	50	27	15	14	14
284B	100 grams/dose	50	46	15	12	11
284C	control	50	41	6	6	6
GLAND (HOG) EQUIVALENT						
100C	50 grams/dose	50	44	26	22	22
100D	control	50	33	16	12	12
100H	50 grams/dose	50	39	27	24	23
100G	control	50	34	19	14	14
199A	50 grams/dose	50	41	17	17	16
199B	control	50	25	13	10	10

than among the control animals. However, the observed differences were usually small and there was overlapping between the numbers of treated and control animals which survived among different groups. When large doses of extracts from hog adrenal glands were given the incidence of survival was significantly greater than among the control animals. The compound 11-desoxycorticosterone acetate was ineffective in the doses tested.

Histologic changes in the adrenal cortices were not studied in these experiments. Gross inspection showed small hemorrhagic areas in the capsules of the adrenal glands and within the gland. Hemorrhage and ulceration were observed in the stomach and small intestine. All of the intraabdominal organs showed a marked hyperemia following the injection of peptone.

DISCUSSION. There have been a number of studies on the effect of adrenal cortical extracts and hormones on the resistance of animals and patients to stress. The lack of agreement in results and conclusions has been reviewed by Swingle and Remington (2). One of the ultimate objectives of such studies has been to determine whether or not the cortical hormones are of value in preventing or treating any of the forms of shock which occur in human patients. Since the intraperitoneal injection of peptone is not a natural hazard the results of the

TABLE 2

Number of rats surviving the intraperitoneal injection of 5.5 cc. of peptone with and without treatment with 11-desoxycorticosterone acetate

SOLUTION	CONCENTRATION	NUMBER TESTED	HOURS FOLLOWING INJECTION OF PEPTONE			
			7	24	31	48
271A	control	50	44	26	14	13
271B	Doc-Ac 1 mgm./dose	50	47	23	11	9
272A	Doc-Ac 1 mgm./dose	50	39	12	12	11
272B	Control	50	41	16	7	7
272A	Doc-Ac 0.5 mgm./dose	50	36	19	11	10
272B	Control	50	40	19	12	9
100A	Doc-Ac 2 mgm./dose	50	45	20	15	8
100B	Control	50	39	22	14	11

present study are not represented as a vector in evaluating the possible clinical usefulness of the cortical hormones.

The positive results obtained in this study do justify further studies of shock as produced by naturally occurring damaging agents, especially those which have been described as causing degeneration in the adrenal cortex (3). It may be reasonable to assume that a damaged gland is unable to meet its requirements for secretory activity as effectively as does an undamaged gland.

The most effective results were obtained by the use of extracts of hog adrenal glands. It was shown by Kuizenga et al. (4) that hog adrenal glands contain approximately twice as much biologic activity per unit weight of glands as do beef adrenal extracts. The amount of hog adrenal extract administered daily (two doses per day) represented the activity equivalent of 200 grams of beef adrenal gland.

It will be noted that the numbers of animals used in these experiments were very large. It is our experience that the variability in incidence of survival

among rats subjected to any type of shock is so great even under the most rigidly controlled experimental conditions that studies on small numbers of animals are of uncertain value.

SUMMARY

Normal male rats of 185 to 195 grams weight were caused to develop shock by the intraperitoneal injection of a 50 per cent solution of peptone. A comparison was made in the incidence of survival up to forty-eight hours of rats which were treated with cortical extracts, 11-desoxycorticosterone acetate and rats given control solutions. The injection of large doses of cortical extracts increased the incidence of survival over that of untreated animals. Extracts derived from hog adrenal glands were superior to extracts of beef adrenal glands. The administration of 11-desoxycorticosterone acetate and of small doses of adrenal cortical extracts was ineffective.

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REFERENCES

- (1) INGLE, D. J. This Journal 139: 460, 1943.
- (2) SWINGLE, W. W. AND J. W. REMINGTON. Physiol. Reviews 24: 89, 1944.
- (3) MOON, V. H. Shock, its dynamics, occurrence and management. Lea and Febiger, Philadelphia, 1942.
- (4) KUIZENGA, M. H., A. N. WICK, D. J. INGLE, J. W. NELSON AND G. F. CARTLAND. J. Biol. Chem. 147: 561, 1943.

THE EFFECT OF VARIOUS CONDITIONS ON THE RESPIRATION OF RAT HEART MUSCLE IN VITRO

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Mammalian heart muscle cannot tolerate anaerobic conditions. Clamping of a branch of the coronary artery immediately renders the heart hypodynamic (Wiggers, 1934) and compensation, if it occurs, is due to the increased activity of the unaffected ventricular fibres. Moreover, the area from which the blood has been excluded does not recover its contractility if anaerobic conditions are maintained for as short a period as five minutes. In this respect heart muscle differs from skeletal muscle and all other tissues with the possible exception of brain. It seems evident that irreversible changes rapidly take place in heart muscle. In order to investigate the possible nature of these changes the respiration of heart muscle slices was studied. Slices were made from heart immediately after its removal from the body and compared with slices made from hearts allowed to incubate semi-anaerobically for varying lengths of time at different temperatures. Because comparatively little work has been done on heart muscle slices, the effect of ions and certain drugs on the respiration was also studied.

EXPERIMENTAL. Young adult rats kept on stock diet were used. They were killed by a blow on the head, the hearts removed and immediately sliced with a sharp razor transversely across the ventricles so that rings approximately 0.2 mm. thick were formed. These were placed in shallow dishes containing well-oxygenated Krebs-Henseleit (1932) solution without glucose in which phosphate was substituted for bicarbonate. The time between the removal of the heart from the body and the completion of the slicing was five minutes. The slices were trimmed and weighed and usually 5 or 6 (100 mgm. wet weight) were placed in each Warburg vessel containing 2.0 ml. of the same solution. The oxygen uptake was measured at 37° in air. Every experiment was run either in duplicate or triplicate. The results checked to within 15 per cent. For the incubation experiments hearts were removed from the animal and placed in the solution for different periods of time at either 37° or 5° before slicing.

Figure 1 shows that the oxygen uptake of slices made immediately upon removing the heart from the animal is constant over a period of 4 to 5 hours. This indicates that the cell structure remains intact under these conditions and that there is no loss by diffusion or destruction of essential cell elements such as co-enzymes and substrates. The respiration of slices made from hearts incubated from 5 to 15 minutes at 37° is however definitely depressed and the rate falls off with time. It is interesting that as short a period as 5 minutes should produce a definite effect, despite the fact that some blood is left in the coronaries. On the

other hand, as shown in figure 1, it is possible to incubate the heart for 30 minutes at 5° without affecting the subsequent respiration of the slices. Liver or kidney slices, however, can be incubated for at least 20 minutes at 37° under the same

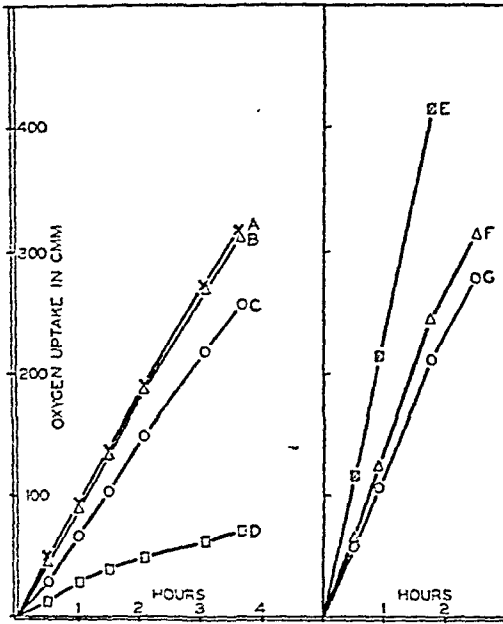


Fig. 1

Fig. 1. A: heart sliced after 30 minutes at 5° ; B: heart sliced immediately; C: heart sliced after 5 minutes at 37° ; D: heart sliced after 15 minutes at 37° ; E: kidney sliced immediately and after 20 minutes at 37° ; F: liver sliced immediately; G: liver sliced after 20 minutes at 37° .

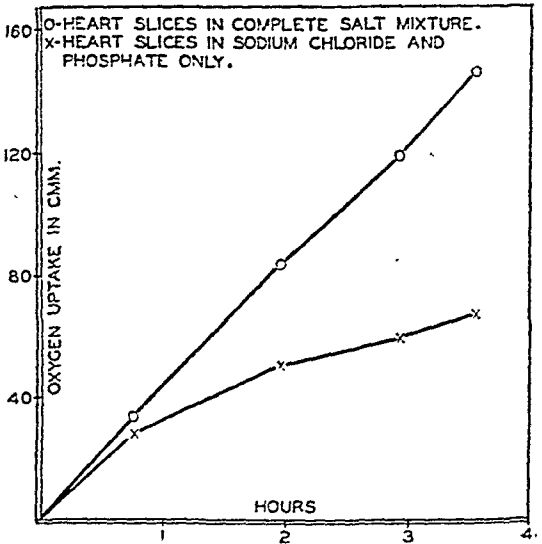


Fig. 3

Fig. 3. The heart was incubated 20 minutes at 21° before slicing.

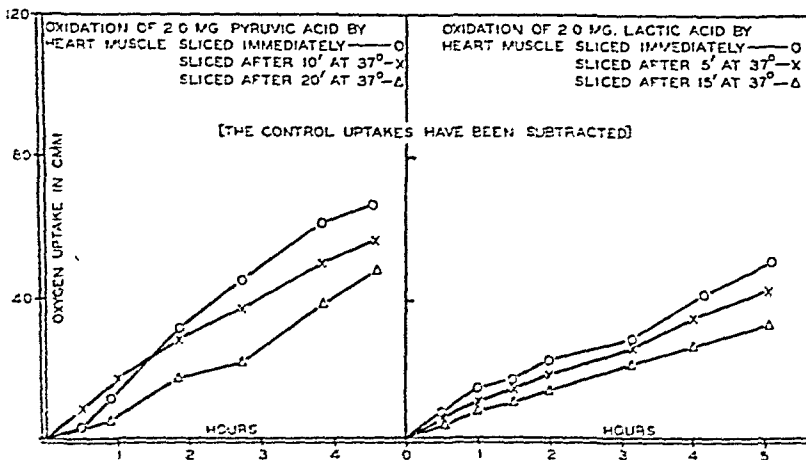


Fig. 2

conditions without affecting the subsequent respiration. Preparations of brain and skeletal muscle present technical difficulties which make the comparison of these tissues with the heart unreliable. These results show that the respiratory mechanism of heart muscle is very sensitive to anaerobic conditions.

The following substrates were added to heart muscle slices in order to determine whether they affected the oxygen uptake: glucose, Cori ester, Embden ester, mannose, adenylic acid, glutamic acid, alanine, acetic acid, tyramine, fumaric acid, succinic acid, lactic acid and pyruvic acid. Only the last three had an effect. Succinic acid is oxidized at an equal rate by slices cut from a fresh heart or from one incubated for 30 minutes at 37°. This indicates that the depressed oxygen uptake of incubated heart is not caused by damage to the succinic acid dehydrogenase or to the cytochrome-cytochrome oxidase system. Addition of lactic or pyruvic acids to slices from a fresh heart has a variable effect, but usually causes an increase in the oxygen uptake. This variability might be expected if it is assumed that under these conditions the oxidation of lactic and pyruvic acids contributes to the overall oxygen uptake. The effect of adding these substrates would then depend on whether or not the enzymes responsible for their oxidation are already saturated by these acids supplied from carbohydrate precursors. If these compounds are added to slices from a heart which has been incubated from 5 to 15 minutes at 37° before slicing, they will also increase the oxygen uptake. In spite of the considerable reduction in the control uptakes caused by incubation, the oxidation of lactic and pyruvic acids is only slightly decreased. These results are shown in figure 2. Pyruvic acid was estimated by the method of Clift and Cook (1932) after it had been shaken with, and apparently oxidized by slices made from heart incubated 10 minutes at 37°. From the oxygen uptake it was calculated that 0.48 mgm. of pyruvic acid was oxidized; from the carbon dioxide production, 0.47 mgm. was decarboxylated; and from the chemical determination 0.44 mgm. had disappeared. This indicates that the increased oxygen uptake is caused by the actual oxidation of pyruvic acid and not by a non-specific salt effect. This is probably also true of lactic acid although no direct chemical estimations were made. These results suggest that the depressed oxygen uptake of slices from incubated hearts is not due to the destruction of the lactic and pyruvic acid dehydrogenases, coenzyme I or thiamine pyrophosphate. Govier (1944) has shown that there is little destruction of coenzyme I and thiamine pyrophosphate in dog heart one hour post mortem.

In order to determine whether the oxidation of fatty acid was damaged by anaerobic incubation, the R.Q. of slices made from fresh heart and heart incubated for 15 minutes at 37° was measured. The average value obtained for the former was 0.86 (0.82–0.89) and although the oxygen uptake of the latter was depressed 60 per cent, the R.Q. was 0.85 (0.80–0.89). This indicates that anaerobic incubation depresses the fatty acid and carbohydrate oxidation to an equal extent.

The effect of cations. The removal of either potassium, calcium or magnesium from the surrounding fluid has little or no effect on the oxygen uptake of heart slices. If all three cations are removed together, leaving only a mixture of sodium chloride and sodium phosphate, the oxygen uptake is markedly depressed. This is shown in figure 3. This depression is not overcome by the addition after 30 minutes of the three missing cations in the proper proportions. The damage induced by their removal occurs rapidly and is irreversible. The same depres-

sion occurs if phosphate is omitted and the slices suspended in pure isotonic sodium chloride and the pH adjusted to 7.4. It is thus not possible to correlate the effects of the removal of these cations on the contraction of the heart with the effects of their removal on respiration. Slices suspended in sodium chloride and sodium phosphate are still able to oxidize succinic, lactic and pyruvic acids, and have the same R.Q. as slices suspended in the complete salt mixture.

The effects of chloral hydrate and avertin. Halogenated organic compounds are often toxic to the heart and their use is contra-indicated in heart disease. It was therefore of interest to study their effects on the respiration of heart slices. It was not possible to use chloroform because of the difficulty of maintaining a constant concentration. Chloral hydrate and avertin were therefore used. These compounds inhibit the oxygen uptake of slices from both fresh and incubated hearts, the effect on the latter being somewhat greater. Thus chloral hydrate produced a 45 per cent inhibition of the oxygen uptake of slices from a fresh heart and a 67 per cent inhibition of the uptake of slices from a heart incubated for 15 minutes at 37°. Avertin and chloral hydrate are about equally ef-

TABLE 1

The effect of chloral hydrate on the oxygen uptake of slices from unincubated heart and the oxidation of lactic and pyruvic acids

TIME	CONTROL	2.0 MGM. LACTATE	2.0 MGM. PYRUVATE	CHLORAL HYDRATE	CHLORAL HYDRATE 2.0 MGM. LACTATE	CHLORAL HYDRATE 2.0 MGM. PYRUVATE
<i>min.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>
40	38	41	40	25	41	37
80	79	85	82	46	85	77
120	116	128	121	65	128	115
180	165	184	171	91	183	166
240	207	236	210	113	230	207

fective in equimolar concentrations. Neither drug is effective in concentrations less than M/1000, and both reach an apparent maximum at M/300, after which further increase in the concentration has relatively little effect. Neither drug interferes with the oxidation of succinic, lactic or pyruvic acids. This is shown for chloral hydrate and the two latter acids in table 1. In this experiment the addition of lactic and pyruvic acids had little effect on the control uptake of slices from fresh heart. Chloral hydrate inhibited the control uptake about 50 per cent, but in its presence the two acids restore the uptake. These drugs do not change the R.Q., and thus their action resembles that of anaerobic incubation and lack of cations. M/1000 moniodoacetic acid inhibits the oxygen uptake of slices 75 per cent, and the oxidation of lactic and pyruvic acids completely. M/50 sodium fluoride has a similar effect.

DISCUSSION. Heart muscle slices made rapidly from a rat heart just removed from the animal apparently have a normal respiratory metabolism. The criterion for this is that the oxygen uptake remains constant over a comparatively long period of time, and that the R.Q. of 0.86 is identical with that found by

Clark, Gaddie and Stewart (1931) for the perfused frog heart. Short periods of anaerobiosis definitely depress the respiration if the heart is incubated at 37°. If this period of incubation is extended to 15 or 20 minutes the heart is usually in systolic contraction. During this time lactic acid is probably accumulating. But part at least of this lactic acid must be washed out when slices are cut from such a heart, because the addition of more to the slices always increases the oxygen uptake. Since of all the substrates tried only succinic, lactic and pyruvic acids increase the oxygen uptake of slices from fresh and incubated heart, it is only possible by this method to study the effects of anaerobiosis on the oxidation of these three compounds. The evidence indicates that anaerobiosis disturbs the carbohydrate metabolism at some stage between the hexose phosphates and lactic and pyruvic acids. But since the R.Q. remains the same, depression of fatty acid oxidation must also occur. Lack of certain cations, or the presence of chloral hydrate or avertin apparently produces changes similar to anaerobiosis and this suggests that injury produced by a number of different agents may disturb certain catalysts or structural relationships which result in a depression of respiration.

SUMMARY

1. The respiration of rat ventricle slices made as rapidly as possible from heart just removed from the animal is constant over a period of at least 6 hours. The average R.Q. is 0.86.

2. If the heart is incubated for 5 to 20 minutes at 37° before slicing the respiration is depressed, the extent depending on the time of semi-anaerobic incubation. At 5° it is possible to incubate the heart for 30 minutes without depression of the subsequent respiration.

3. Slices made from hearts incubated at 37° still oxidize succinic, lactic and pyruvic acids indicating that the cytochrome-cytochrome oxidase system, co-enzyme I and thiamine pyrophosphate are still functioning. The R.Q. remains unchanged.

4. Addition of acetate, alanine, glutamic acid, adenylic acid, fumaric acid, tyramine, glucose, Cori ester, Embden ester, or mannose has no effect on the oxygen uptake of slices from fresh or incubated hearts.

5. The simultaneous absence of potassium, calcium and magnesium depresses the respiration of heart muscle slices irreversibly. Under these conditions succinic, lactic, and pyruvic acids are still oxidized and the R.Q. is unchanged. A similar effect is obtained if M/300 chloral hydrate or avertin is added to the complete salt solution.

REFERENCES

- CLARK, A. J., R. GADDIE, AND C. P. STEWART. *J. Physiol.* **72**: 443, 1931.
CLIFT, P. C. AND R. P. COOK. *Biochem. J.* **26**: 1788, 1932.
GOVIER, W. M. *Science* **99**: 475, 1944.
KREBS, J. A. AND K. HENZELEIT. *Ztschr. Physiol. Chem.* **210**: 33, 1932.
WIGGERS, C. J. *Physiology in health and disease*. Lea and Febiger, Philadelphia, 1934, p. 666.

SOME PROPERTIES OF MAXIMAL AND SUBMAXIMAL EXERCISE WITH REFERENCE TO PHYSIOLOGICAL VARIATION AND THE MEASUREMENT OF EXERCISE TOLERANCE¹

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Exercise experiments or tests may be classed as submaximal or maximal depending upon the response they induce in the subject. The essential criterion is whether the subject is able to complete the task or is forced to quit from excessive fatigue or exhaustion. By proper selection of the intensity and duration of exercise in relation to the physical capacity of the subject, the test may be made submaximal or maximal. When it is desired to plan such tests for a fairly heterogeneous group of subjects, including extremes of high and low fitness, certain considerations apply: *a*, the intensity and duration of submaximal exercise cannot exceed the capacities of the poorest subject; *b*, a maximal exercise test, on the other hand, must bring all subjects to a comparable degree of exhaustion.

Submaximal experiments have been widely reported in the literature. They range from very brief and vigorous work (Gemmell et al., 1931; Knehr et al., 1942) to very moderate work in which a steady state can be attained (Taylor, 1941), subject only to the rule that the man can complete the work in the pre-arranged time. No great difficulty is met in devising a submaximal exercise, since one need only determine what intensity and duration is submaximal for the weakest subject. Somewhat more difficulty is encountered in designing a satisfactory maximal test for a heterogeneous group of subjects. Two main types of maximal test have appeared in the literature: *a*, work to exhaustion or fatigue with a work-load initially set near the subject's limit of capacity (Knehr et al., 1942; Robinson and Harmon, 1941); or *b*, a graded series of work-loads bringing the subject gradually to the point of exhaustion (Briggs, 1920; Schneider, 1931; Taylor, 1941). In all of these, time-run, total work or some equivalent is used as a measure of the performance. The single-work method, however, is inadvisable for a heterogeneous group of subjects, because no matter what the intensity and duration, strong subjects tend to reach a steady state and continue for a relatively long time before becoming sufficiently fatigued to stop,

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while weak subjects tend to become exhausted before second wind is reached. A distribution of performance scores, obtained in such an experiment, displays a marked positive skewness. On the contrary, this tendency is avoided in the graded-series type of experiment which, with heterogeneous groups of subjects, gives an approximately normal distribution of performance scores. These specifications for submaximal and maximal tests have been carefully followed in the present series.

It is the aim of this paper to compare responses of a group of subjects to submaximal and maximal work in repeat experiments, to compare intra-individual with inter-individual variation, and to point out some essential differences in fitness as tested in these types of experiments.

Subjects. Thirty-one subjects, ranging in age from 19 to 26 (except one, 33), were given the treadmill test twice, about 3 days intervening between tests. The group contained two athletes in training, but otherwise was made up of normally active college students, and except for the two athletes no particular selection was made of extremes in fitness. All subjects were medically certified as free from defects which might affect their exercise tolerance.

Tests. The repeat test routines were given either in morning or afternoon, but always at the time of day corresponding to the first test. Subjects were instructed to eat only a light meal at the mealtime preceding their test, and no experiment was scheduled within $1\frac{1}{2}$ hours of mealtime.

The routine, which included both submaximal and maximal exercise, was as follows:

The walk (submaximal): 108 meters per minute; grade 5 per cent; duration 4 minutes.

Interim rest: Sitting 4 minutes.

The run (maximal): 162 meters per minute; grade initially set at 5 per cent but elevated 1 per cent each minute; duration, time to exhaustion of the subject.

Recovery: Sitting 15 minutes.

Thus, speed, duration and grade were constant during the walk, and speed was constant during the run, but the grade was periodically increased (without interrupting the experiment) during the run and duration was a function of the fitness of the subject. Time to the nearest quarter-minute (time-run) was taken as the performance measure of fitness. Subjects were motivated to reach a true state of exhaustion by preliminary instruction and exhortation, and encouragement during the run.

Physiological measurements. Heart rates were recorded graphically throughout the experiment by use of the Henry electrocardiotachometer. Respiration rates were recorded graphically with a marker circuit operated by a gate-valve switch placed in the expired airway. Expired air was collected during the walk and run with a face mask and a double-chamber 400 liter Krogh-type gasometer, from the gauge of which readings of ventilation were made each minute. However, submaximal ventilation was calculated as the average per minute ventilation during the period $1\frac{1}{2}$ to 4 minutes of walk, and maximal ventilation is that in the last minute of the run. If the run terminated at some fraction of a

minute, the complementary fraction was used to calculate the portion of the preceding minute's ventilation, which was added to that in the last part minute, in order to give the best estimate of ventilation during the last whole minute in the run. Samples of the expired air, drawn from the expired air collections described for the submaximal and maximal ventilations, were determined in duplicate by Haldane analyzers. Hence, percent oxygen and CO_2 , and oxygen consumption correspond to the submaximal and maximal ventilations. Lactates were determined from capillary blood drawn 2 minutes after the walk, and 5 minutes after the run by the colorimetric method of Barker and Summer-son (1941).

The performance criterion of fitness. The only defensible *a priori* criterion of the fitness of a man for heavy physical exercise is the amount of such work the man can do. Such a fitness criterion is provided by time-run (to exhaustion), for which the data are:

	N	Mn	S	RANGE
First test.....	31	8.0	2.01	4.5 - 12.5
Second test.....	31	8.4	2.37	4.5 - 13.0
Average.....		8.2	2.23	

These figures show that the subjects as a group tended strongly to duplicate their first performances, although there was a mean gain of almost one-half minute. In spite of the fact that they had no simple way of knowing the time during the run, and therefore could not deliberately plan to repeat their performances, the individual consistency was very high. The reliability coefficient (r_{11}) for the 31 cases was 0.95. Time-run in the maximal treadmill test may accordingly be justified as a criterion of fitness on its "face validity," its high reliability, and on additional physiological evidence presented below.

Sources of variation in physiological measurements. In human, as in all biological material, measurements of a given function have a certain error or variation. An infinite number of measurements would be required to approximate the true value, but practically one must be content with a few measurements which will provide an estimate of the variation so that one can define the confidence which may be placed in an obtained value.

The following scheme defines the relationship between sources of variation as determined for the present data:

$$\text{total variation } (S_T) \quad \left\{ \begin{array}{l} \text{test-retest variation } (S_D) \\ \text{variation in method } (S_M) \\ \text{intra-individual variation } (S_P) \\ \text{inter-individual variation } (S_G) \end{array} \right.$$

Estimations of method and test-retest variations have been made from the duplicate series using the formula:

$$\text{S.E.} = S\sqrt{1 - r_{11}}$$

where S is the average standard deviation for the two series and r_{11} is the correlation between the duplicate series.²

Calculations of S_M were made from duplicate series of determinations from split samples in the cases of per cent oxygen and CO_2 , and blood lactate. That for heart rate was determined by correlating series of simultaneous stethoscope and electrocardiotachometer counts. The error of ventilation was estimated from calibration figures, and that for respiration rate was assumed to be one-half a respiration. The error for oxygen consumption was calculated from the combined errors of ventilation and per cent oxygen. The errors for ventilation, oxygen consumption and respiration rate, which alone were not directly determined, have been made conservatively large.

Since two tests were made on each individual, S_D was similarly calculated to express test-retest variation. Here, r_{11} is the conventional test-retest reliability coefficient, combining variation due to method and that due to unaccounted day-to-day changes in the individual. It should be stressed that both tests were identical in the duplicate series, and well defined changes in the fitness of the subjects, such as those due to illness, fatigue, etc., from test to retest were excluded. Then, since no correlation need be assumed between S_D and S_M :

$$S_P = \sqrt{S_D^2 - S_M^2} = \text{the net intra-individual variation.}$$

Since the total variation is known from the standard deviations of the measures for the entire group, one may similarly calculate:

$$S_G = \sqrt{S_T^2 - S_D^2} = \text{the net inter-individual variation.}$$

The data are given in table 1.³ In all cases where duplicate pairs of values were obtained, these were averaged for use in the subsequent treatment. Thus, duplicate samples of oxygen, CO_2 and blood lactate were averaged to give the values considered characteristic of that experiment. Corresponding test-retest measures on the individual were averaged for all functions to yield a measure considered to be characteristic of the individual for intercorrelation between the functions and with the criterion. The data in table 1 show, first of all, that variance due to method is usually less than one per cent of the total variance, and even in the case of submaximal blood lactate (6 per cent) it is far less than the other two classes of variance. Most noteworthy is the generally large intra-individual variance. Since test reliability is an inverse function of the combined variances of method and intra-individual, it is clear that the latter is by far the greatest source of test unreliability. The respiratory measures taken during the walk, with the exception of respiration rate, show about the same

² This formula gives the standard error of a measurement (Peters and VanVoorhis, 1940). The distinction between r_{11} , the correlation between different forms of the same test, and r_{11} , the correlation between two administrations of the same test, which Kelley (1924) has stressed for mental tests is hardly applicable for physiological tests where elements of voluntary control and learning are negligible. $\sqrt{r_{11}}$ may therefore be considered an estimate of the correlation between an obtained measure and the "true" measure (the mean of an infinite number of observations). $S\sqrt{1 - r_{11}}$ is the standard error.

³ Variation has been converted to variance (S^2) in expressing percentages.

intra-individual as inter-individual variance, but for lactate and heart rate the ratio is about 1:4. In maximal exercise, however, there is a substantial gain in the inter-individual variance of the respiratory measures, with the exception of ventilation which, with heart rate and blood lactate, shows essentially unchanged proportions.

The foregoing comparison of variances is useful in a number of ways. It directs attention to the large intra-individual variation which is the greatest source of test unreliability because in the usual situation it serves only to detract from the differentiating effectiveness of the test. Such intra-individual variation, unaccompanied by any tangible change in the fitness of the individual,

TABLE 1

SUBSCRIPT	MEASURES	MEAN	RANGE	CORRELA- TIONS		S_T	STANDARD ERRORS		PER CENT OF TOTAL VARIANCE		
				r_{11}	r_{12}		Test-retest S_D	Method S_M	Method S_M/S_T	Intra-Ind. S_P/S_T	Inter-Ind. S_G/S_T
Submaximal Exercise											
(1)	Time-run (minutes)	8.2	4.5- 13.0	0.95							
(2)	Heart rate (beats/min.)	130.0	108 -153	0.78	-0.56	11.18	5.29	0.87	0.6	21.8	77.6
(3)	Blood lactate (mgm %)	32.2	13 - 61	0.77	-0.44	11.44	5.44	2.80	6.0	16.6	77.4
(4)	Resp. rate (resp/min.)	24.3	13 - 35	0.62	-0.21	5.35	3.28	0.50	0.8	36.8	62.4
(5)	Ventilation (liters/min.)	32.3	22 - 40	0.49	-0.50	3.63	2.59	0.20	0.3	50.5	49.2
(6)	Per cent oxygen	5.20	4.5- 6.4	0.56	0.34	0.438	0.289	0.026	0.4	43.2	56.4
(7)	Per cent carbon dioxide	4.30	3.9- 4.9	0.42	0.06	0.273	0.208	0.026	0.9	58.2	40.9
(8)	Oxygen consum. (liters/min.)	1.65	1.2- 2.0	0.59	-0.20	0.169	0.108	0.013	0.3	40.2	59.5
(9)	Body weight (kilos)	73.8	60 - 91		-0.05						
Maximal Exercise											
	Heart rate	198.1	188 -212	0.81	0.01	7.31	3.19	0.87	1.4	17.6	81.0
	Blood lactate	217.6	117 -328	0.71	-0.14	57.0	30.9	10.3	3.2	26.1	70.7
	Resp. rate	49.6	32 - 62	0.89	-0.06	7.43	2.43	0.50	0.4	10.2	89.4
	Ventilation	104.1	79 -125	0.53	-0.06	10.94	7.47	0.20	0.03	46.6	53.4
	Per cent oxygen	3.38	2.5- 4.1	0.81	0.45	0.426	0.185	0.032	0.6	18.3	81.1
	Per cent carbon dioxide	3.98	3.1- 4.6	0.70	0.44	0.404	0.220	0.027	0.4	29.2	70.4
	Oxygen consumption	3.48	2.8- 4.1	0.70	0.39	0.478	0.264	0.013	0.1	30.4	69.5

must at the present time be considered an obstacle to the reliable determination of the prevailing fitness of the individual. Most of the measures, which are of low reliability in the submaximal exercise, are improved when taken during maximal exercise. Ventilation, however, is of low reliability in both forms of work. It is clear that in the search for measures of fitness as much attention must be given to reliability of measures as to intrinsic validity.

Comparison of responses to submaximal and maximal exercise. Determinations of resting functions were omitted from this study because other unpublished observations in this laboratory indicate very low correlations between the functions studied and measures of exercise tolerance. In general, this is borne out by the literature (see, for example, Knehr et al., 1942).

The mean values in table 1 show the submaximal exercise to be in the category

of "hard work" by Dill's (1936) standard. Heart and respiration rates and blood lactates are about double, and oxygen consumptions and ventilations are over four times commonly accepted resting values. Maximal values for heart and respiration rates are 3 to 4 times resting, while those for ventilations, oxygen consumptions and lactates are 13 to 14 times resting. These averages are slightly higher than those for young men in exhausting work summarized by Robinson (1937).

Variations about these mean values, however, are very striking as shown by the ranges and standard deviations (S_T) in table 1. Lactates are most variable both in submaximal and maximal exercise. The significance of these dispersions has been discussed above in relation to method, intra-individual and inter-individual sources. We now concern ourselves with this variation from the standpoint of inter-correlation between the measures and with the time-run criterion.

The possibility of predicting time-run from submaximal measures may be examined in the zero-order correlations with the criterion (table 1). In general, these correlations, which range from -0.56 for heart rate to 0.06 for per cent CO_2 , indicate a tendency in the fitter subjects to perform the submaximal work at a lesser "physiological cost." The less fit men had higher heart rates, respiratory rates, blood lactates, ventilations and oxygen consumption. The extent of these differences, however, is not great and oxygen consumption, representing gross mechanical efficiency, is only slightly higher in the weaker subjects. Per cent oxygen, an index of the efficiency of respiration, correlates positively with time-run while body weight and per cent CO_2 are insignificantly correlated with the criterion. Respiratory quotient, which under these circumstances is too strongly affected by blood gas displacement to be representative of catabolism, has been evaluated by the semi-partial correlation.⁴ The correlation is -0.27 , showing a slight tendency toward greater CO_2 displacement in less fit subjects.

Of the submaximal measures, only three have high enough correlation with time-run to offer promise of a prediction; they are heart rate, blood lactate, and ventilation. The possible multiple correlations, involving these three variables and the criterion, have been computed with the following results:⁵

$$R_{1(23)} = 0.57$$

$$R_{1(25)} = 0.63$$

$$R_{1(35)} = 0.58$$

$$R_{1(235)} = 0.63$$

⁴ The Franzen (1928) semi-partial states the r between two measures when the influence of a third has been removed from one of them. Since we know the correlations between per cent O_2 and per cent CO_2 and the criterion, we are here interested in the properties of their ratio. This is best treated by correlating CO_2 , from which the influence of O_2 has been removed, with time-run.

$$\text{Thus, } r_{(x \cdot y)z} = \frac{r_{xz} - r_{yz} \cdot r_{xy}}{\sqrt{1 - r_{xy}^2}}$$

where, x = per cent CO_2

y = per cent O_2

z = time-run.

⁵ See table 1 for subscripts.

Since heart rate and ventilation alone yield a multiple of 0.63 it is clear that lactate does not add significantly to the battery because when it is included the multiple does not rise. A multiple correlation of 0.63 will not satisfactorily predict the criterion when it is considered that the standard error of such prediction, $(S_1\sqrt{1-R^2})$, is 1.7 minutes, 58 per cent of the variance of time-run.

In contrast to submaximal the responses to maximal exercise tend to be insignificantly correlated with time-run. This is true for heart rate, respiratory rate, ventilation and blood lactate. The evidence is that each subject ran to his individually peculiar maximum value, which has little relation to fitness. The length of time he was able to run is the essential variable. On the other hand per cents O_2 and CO_2 and oxygen consumption in maximal work show low but positive relationships with the criterion. The change in these relationships from submaximal to maximal exercise is instructive. Per cent O_2 rose from 0.34 to 0.45, per cent CO_2 from 0.06 to 0.44, and oxygen consumption from -0.20 to 0.39. This last finding is of particular interest. In submaximal exercise the correlation between oxygen consumption and body weight was 0.71, and with weight partialled out, $r_{(8.9)1}$ is -0.23, but slightly higher than r_{18} . In maximal exercise the oxygen consumption correlation with weight drops to 0.43 and $r_{(8.9)1}$ rises to 0.46 and changes sign. This means that in submaximal exercise oxygen consumption is chiefly a function of body weight, and only slightly related to fitness as shown by the small negative correlation with the criterion, but in maximal exercise the relation with weight drops markedly and the correlation with the fitness criterion increases very considerably. Those subjects, regardless of body weight, who were able to run longest were those who could reach higher oxygen consumptions in spite of no higher ventilations, heart and respiratory rates, and blood lactate accumulations. They were able to transport and consume oxygen at higher rates.

Figure 1 displays the average heart rate for four groupings of the subjects selected from quartiles of the time-run distribution. The most noteworthy feature in this figure is that the shape of these curves during the run is the only valid differential between the heart rates of the four groups. It is true that group I, composed of the fittest men, has a lower average heart rate throughout, but at several points in the experiment the other groups change rank order of mean heart rate. Only during the run are the curves segregated in proper rank order, but even here if the data are treated without regard to grouping, the correlation between absolute heart rates and the criterion is only moderate, i.e., this correlation at three minutes of running, which is the latest point at which all cases can be included, is -0.56. Similar correlations at other points in the routine are as follows: walk, -0.56; 2 minutes after walk, -0.21; 5 minutes after run, -0.14; and 15 minutes after the run, -0.09. Absolute levels of heart rate therefore are of limited validity.

The trend of heart rate during the run, however, does offer valid relationships. Cubic equations⁶ have been developed for each of the experiments on 29 sub-

⁶ Of the type: $Y = a + bX + cX^2 + dX^3$ where the ordinate (Y) is heart rate and the abscissa (X) is time from the beginning of the run.

jects, (2 cases were eliminated because of incomplete heart rate data) and various functions of the fitted curves correlated with time run. The most striking difference between high and low fitness subjects is the lower, flatter, and straighter curves of the former. Acceleration at the 2nd minute of the run, expressed by the second derivative of the cubic fit, comprises an index of the extent to which the high ranking men displayed a lesser change in the rate of increase of heart rate.⁷ This function, combined with heart rate at the mid-point of the run and submaximal heart rate, provides a highly valid battery.

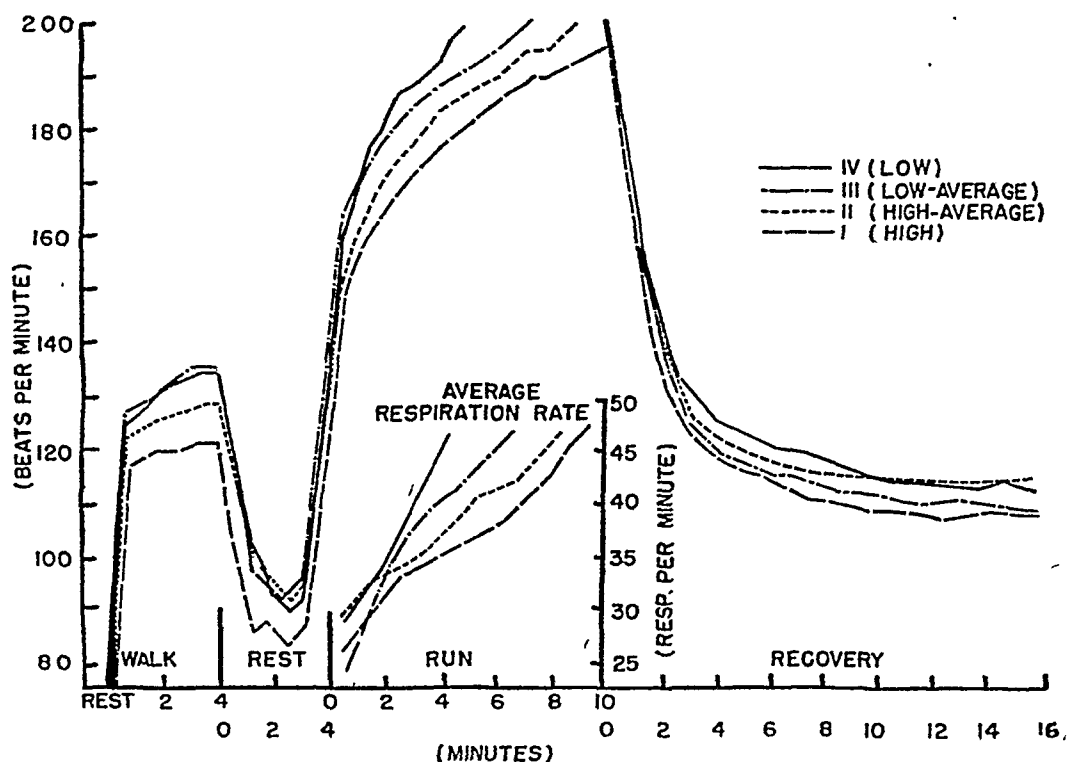


Fig. 1. Average heart rates for four fitness groups.

The zero-order and multiple correlations and regression equations are given in the following tabulation:

SUBSCRIPT	MEASURE	CORRELATION WITH TIME-RUN (1)		STANDARD REGRESSION COEFFICIENTS	
		1st test	2nd test	1st test	2nd test
(2)	Acceleration	0.77	0.78	0.7264	0.7778
(3)	Submaximal rate	-0.47	-0.53	-0.5781	-0.5151
(4)	Rate at midpoint	-0.08	-0.11	0.2894	0.4782
	$R_1(23)$	0.87	0.82		
	$R_1(234)$	0.90	0.91	(inherent regressions)	
	$R_1(234)$	0.89	0.89	(alien regressions)	

⁷ This function is negative in all cases, that is, all of the men showed a decrease in the rate of heart rate acceleration at the 2nd minute. But the fitter subjects had a lesser slope in heart rate (first derivative) and a lesser negative change in this slope (2nd derivative). The double negative has for the sake of simplicity been dropped and acceleration, the change in slope, given a positive sign.

It is noted that the three measures yield a multiple correlation of about 0.90 with the time-run criterion, accounting for 82 per cent of the variance in the latter function. The stability of the regression coefficients is best judged by applying them to alien samples of data. In the present case the coefficients computed for 2nd test data were applied to 1st test data, and vice versa. Such alien regressions deliver substantially as high multiples as the inherent regressions, attesting to the stability of the inter-relationships from experiment to experiment. The reliability coefficient for this battery (r_{11}) is 0.80 and when one extremely divergent case is removed, it rises to 0.90.

Mean curves of respiration rate for the four groups, shown in figure 1, display the same general dispersion as heart rate, but tend to increase linearly after two minutes of running. The respiratory rate of the fitter subject increases at a slower rate, but reaches about the same maximal value as the unfit subject, as will be recalled from the insignificant correlation between maximal respiratory rate and time-run. Curves for ventilation during the run have not been reproduced here because they are irregular and the segregation between the groups is not clearcut. That for group IV rises steeply to the maximum, but the other groups have practically superimposed curves diverging irregularly in the last minutes of the run.

The physiological interpretation of the differences in response between those who ran for only a short time and those who reached large outputs is in general outline fairly simple. Since the work was gradually increased throughout the run no one could attain an absolute steady state. Rather subjects reached relative degrees of adaptation which permitted them to continue for varying lengths of time. Weak subjects tended rapidly to excessive ventilation, respiratory and heart rates, and blood lactates while strong subjects were able to attain, at least during early minutes of the run, a relative state of adaptation which postponed the approach to maximal and exhausting levels of function. These relations are best shown by the heart rate trends where the mathematical proof is convincing.

DISCUSSION. The difference in time-run between subjects, as far as oxygen consumption is concerned, can be explained on the basis of greater circulatory reserve. If in respect to oxygen transport we consider heart rate, stroke volume and arterio-venous oxygen difference to be the first, second and third factors in circulatory reserve, maximal heart rate, which does not correlate with the criterion, can at once be eliminated as a variable, and attention centered upon differences in stroke volume and arterio-venous oxygen difference. It has been shown by Henderson et al. (1927) that increase of stroke volume on exercise is least in non-athletes, intermediate in an "active" group, and pronounced in competitive athletes, and Christensen (1937) has reported a very significant increase in stroke volume in one subject after a period of physical training. Recently, Liljestrand et al. (1938) found that while stroke volume rose from 78 to 88 cc. in a group of 12 subjects as work-load was increased from 720 to 1260 kgm-min., two of these subjects whose stroke volumes did not so increase also did not undergo increase of oxygen consumption. Regarding arterio-venous oxygen difference, these authors found no appreciable increase of this function

in the heavy over the moderate work where it had already reached the value of 147 cc. per liter. This indicates that the peripheral circulatory adjustments approach a maximum in moderate work and have little further capacity to increase. The writer knows of no evidence on the extent of individual differences in arterio-venous oxygen difference in exhausting work. One can only speculate on the possible rôles of arterial saturation, and competition between the heat-loss and oxygen-transport duties of the blood in determining critical individual differences in this third factor in circulatory reserve for maximal exercise. But it is quite certain that the second factor, stroke volume, is a significant differential in fitness for exhausting work.

Per cent oxygen removed from the respired air has appeared as an index of respiratory efficiency under various guises in the literature. The "kalorischen Ventilationsquotienten" of Simonson (1926), the "oxygen absorbing power index" of Brice (1939), and the "ventilatory efficiency index" of Barman et al. (1942) are essentially equivalents of this measure. In submaximal exercise per cent oxygen correlates 0.33 with the criterion, but with ventilation partialled out, it is of little significance ($r_{(7.5)1} = 0.14$). In maximal exercise its correlation with the criterion ($r = 0.45$) is slightly increased when ventilation is partialled out ($r_{(7.5)1} = 0.47$). Percent oxygen may therefore be said to have moderate validity when taken under maximal conditions. It seems unlikely that the perfect rank order correlation obtained by Barman et al. between their index and a criterion of fitness could have resulted unless their eight subjects were carefully selected.

The present data may also be used to assess the validity of the "oxygen pulse" as a measure of fitness. This index, first introduced by Henderson and Prince (1914), is the oxygen consumed per heart beat. Recently, Kibler and Brody (1943) have shown that oxygen pulse per unit of body weight is constant within narrow limits over a range of mammals from the mouse to the elephant. They suggest that intra-species variations in the ratio may be used as a measure of "muscular-work capacity." Since in the present data both heart rate and oxygen consumption are correlated with the criterion and we are interested in the quality peculiar to their ratio, again the semi-partial has been used. Values for $r_{(8.2)1}$ are -0.03 and 0.40 for submaximal and maximal work respectively. The former is insignificant and the latter is approximately equal to the validity of oxygen consumption, indicating that the ratio has no independent significance in these data.

Even if it is assumed that the greater oxygen consumption of the fitter subjects in maximal work, indicating greater circulatory reserve, correlates to the extent of 0.55^8 with the criterion, only 30 per cent of the variance has been

⁸ When the correlation between oxygen consumption and time-run is corrected for unreliability, the r rises to 0.55. The formula for this correction is:

$$r = \frac{r_{18}}{\sqrt{r_{11} \cdot r_{88}}}$$

where, r_{12} is the obtained correlation and,
 r_{11} and r_{88} are the reliability coefficients.

explained. There are other basic differences to be discovered. Many of these inhere in the neuromuscular mechanism and do not appear to be represented in the general circulatory and metabolic response. There are qualities of muscular strength and stamina which differ significantly among individuals. As evidence of this, the correlation between a modification of the Behnke "muscle endurance" test,⁹ which was given to our subjects, and time-run on the treadmill is 0.78. Judging by heart rate, and by other subjective and objective signs, this test scarcely taxes the general oxygen transport mechanism, but scores are determined by more localized neuro-muscular endurance. Only when such factors can be determined and brought into the analysis can the theoretical explanation of difference in time-run be fully made.

The very high correlations between heart rate curve functions and the criterion (0.90) indicate that a pattern of cardiac adaptation to exercise has been discovered. This is of greatest practical importance, because it points the way to a practical test of fitness. But the heart rate pattern should be looked upon as a sensitive indicator of the trend of adaptation to the exercise, an integral of many known and unknown differences, affected by respiratory and neuromuscular factors as well as those intrinsic in the heart action itself.

Respiratory and metabolic measurements during rest and recovery have been omitted from the present series, because the literature has been consistent in demonstrating no considerable relationship between fitness and these functions. Knehr et al. (1942) have shown that the recovery curve of heart rate after exhausting work is essentially unchanged by hard physical training. It has been seen that the inter-individual comparisons of heart rate following maximal exercise in the present study (fig. 1) likewise offer no promise for a reliable measure of fitness as determined by time-run. Recovery of heart rate during the first three minutes after the submaximal walk is likewise unpromising. It is true that the group I curve is distinctly lower during this period and is also somewhat lower during recovery from the run, but the lack of consistent differentiation of the other groups invalidates the recovery function, which has been advocated as a measure of fitness by Johnson et al. (1942), Schneider (1920) and Hambly et al. (1925). Similarly Gemmill et al. (1930, 1931) were unable to find reliable differences in recovery ventilation and oxygen consumption after training in light and heavy physical exercise. It seems clear that one must look to the responses *during* exercise, particularly the later phases of adaptation, for critical measures of fitness for hard work, not to the resting or recovery states.

SUMMARY

Thirty-one subjects have been given a test twice, consisting of a four minute walk on the treadmill and a run to exhaustion after a four minute interim rest, the retest following in three days. Heart and respiration rates, ventilation,

⁹ In this test the subject mounts an 18 inch stool at the rate of 40 per minute until no longer able to maintain the rate. The modification consists in adding weights to the subject as the test progresses and repeating it after a two-minute rest. The score is the summed times in the two spells of work.

blood lactate, per cents oxygen and CO_2 , and oxygen consumption were determined during the walk and during the last minute of the run, and the first three of these measures throughout both walk and run. From these data it has been possible to evaluate *a*, the sources of variation in the physiological measures, and *b*, their validity as indicators of fitness.

1. Errors in method were directly determined in four of the measures and calculated from assumptions in the other three. Such errors are less than one per cent of the total variance in all measures except blood lactate.

2. Intra-individual variation, a prominent source of test unreliability, ranges from 10 to 58 per cent of the total variance. In general, this intra-individual variation declines in maximal as compared to submaximal work. Heart rate and blood lactate were the most reliable submaximal measures, but are approximated in maximal work by per cent oxygen and oxygen consumption. Ventilation was of low reliability in both submaximal and maximal work, while respiration rate became highly reliable in the latter form of exercise.

3. Time-run in the maximal test is more reliable than any of the physiological measures, but it is closely approached by a battery of heart rate measures including the second derivative of a cubic fit to the heart rate curve obtained during the run.

4. The highest multiple correlation which could be developed between the best three of the submaximal measures and the time-run criterion was 0.63. Two variables alone, ventilation and heart rate, equal this value.

5. Three heart rate functions, submaximal, rate at midpoint, and the second derivative of the cubic equation, correlate 0.90 with the criterion, and the stability of this relation is proved by cross-application.

6. Heart rate, ventilation, blood lactate and respiration rate at the maximal minute of the run show no significant correlation with time-run, thus indicating that all subjects tended to reach individually varying ceilings, which were unrelated to the time of running. Per cents of oxygen and CO_2 , and oxygen consumption show moderate correlation with time run. This tends to show that fitter subjects were possessed of greater circulatory reserve.

7. The validity of the "oxygen pulse," per cent oxygen, and pulse recovery, as indices of fitness has been seriously questioned, and the conclusion reached that, while 30 per cent of the variance in time-run can theoretically be explained on the basis of oxygen-transport capacity, the remaining variance is yet unaccounted for. The high correlation achieved between time-run and the heart rate battery, which serves as a very reliable index of adaptation to maximal exercise, particularly the later phases of this adaptation, offers great promise for the development of a practical test of exercise tolerance.

REFERENCES

- BARKER, S. B. AND W. H. SUMMERSON. *J. Biol. Chem.* 138: 535, 1941.
BARMAN, J. M., F. CONSOLAZIO AND M. MOREIRA. *This Journal* 138: 20, 1942.
BRICE, A. T. *Am. J. Med. Tech.* 5: 81, 1939.
BRIGGS, H. *J. Physiol.* 54: 292, 1920.
CHRISTENSEN, E. H. *Ergebn. d. Physiol.* 39: 348, 1937.

- DILL, D. B. *Physiol. Rev.* **16**: 263, 1936.
- FRANZEN, R. J. *Ed. Psych.* **19**: 194, 1928.
- GEMMILL, C., W. BOOTH AND B. POCOCK. *This Journal* **92**: 253, 1930.
- GEMMILL, C., W. BOOTH, J. DETRICK AND H. SCHEIBEL. *This Journal* **96**: 265, 1931.
- HAMBLY, W. D., M. S. PEMBREY AND E. C. WARNER. *Guy's Hosp. Reports* **75**: 388, 1925.
- HENDERSON, Y., H. W. HAGGARD AND F. S. DOLLEY. *This Journal* **82**: 512, 1927.
- HENDERSON, Y. AND A. L. PRINCE. *This Journal* **35**: 106, 1914.
- JOHNSON, R. E., L. BROUHA AND R. C. DARLING. *Canad. Rev. d. Biol.* **1**: 491, 1942.
- KELLEY, T. L. *Statistical method*. New York, Macmillan, 1924.
- KIBLER, H. H. AND S. BRODY. *Research Bull. no. 368 College of Agriculture, University of Missouri*, May, 1943.
- KNEHR, C. A., D. B. DILL AND W. NEUFELD. *This Journal* **136**: 148, 1942.
- LILJESTRAND, G., E. LYSHOLM AND G. NYLIN. *Skand. Arch. f. Physiol.* **80**: 265, 1938.
- PETERS, C. C. AND W. R. VANVOORHIS. *Statistical procedures and their mathematical bases*. New York, McGraw-Hill, 1940.
- ROBINSON, S. *Science* **85**: 409, 1937.
- ROBINSON, S. AND P. M. HARMON. *This Journal* **132**: 757, 1941.
- SCHNEIDER, E. C. *This Journal* **97**: 353, 1931.
- SIMONSON, E. *Pflüger's Arch.* **214**: 380, 1926.
- TAYLOR, C. *This Journal* **135**: 27, 1941.

HEMOLYTIC DEPRESSION OF THE ERYTHROCYTE NUMBER BY THE FEEDING OF FAT WITH CHOLINE¹

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It has been shown by Johnson et al. (1, 2) that the feeding of fat to dogs increases the fragility of their red blood cells and causes an increased destruction of erythrocytes as indicated by an increased bile pigment output. The hemolytic agents from fat are presumably soaps and fatty acids which have escaped resynthesis into fat during absorption (3, 4). These workers were unable to detect any anemia due to fat feeding probably because the hematopoietic tissue was able to keep pace with the increased erythrocyte destruction.

The author has shown that the daily feeding of choline hydrochloride to normal dogs for five to seven days had no significant effect on their erythrocyte counts (5). Recently it has been found (6) that the continued daily feeding of choline to dogs for 8 or more days caused a significant depression of the red cell number, which appeared to be due to depression of erythropoiesis. The purpose of this investigation was to see whether choline administration in addition to fat feeding would produce a rapid lowering of the erythrocyte count.

PROCEDURE. Normal dogs were maintained on a basal adequate diet of Purina dog chow and rolled oats. Red blood cell counts, hemoglobin percentage (Hellige), hematocrit determinations, total leukocyte counts and readings of the icterus index were made on blood samples drawn from the saphenous veins of the dogs. Samples were drawn only when the animals were unexcited and in a resting, fairly basal, condition—at least 18 hours after previous feeding or medication.

After normal control values had been determined on the blood, fat and choline feeding was commenced. Fat was given in the form of 60 grams of pure lard daily, which the dogs ate readily for a few days. Choline hydrochloride was administered in dilute aqueous solution by stomach tube in the dosage of 10 mgm. per kgm. of body weight, daily. Four dogs, weighing from 8.7 to 12.2 kgm. were used for this experiment.

Lard alone was fed to three normal dogs, and lard (additionally) was fed to five mildly anemic dogs which had been receiving choline daily for three weeks prior to this procedure.

RESULTS. Figure 1 shows the effects of feeding fat and choline for two to four days to four normal dogs. It will be noted that the erythrocyte numbers of three dogs were reduced by 15 to 27 per cent twenty-four hours after the first dose of fat and choline. Counts were not made on the fourth dog until after the third daily dosage. Hemoglobin and hematocrit percentages were reduced,

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but not quite as greatly as the erythrocyte number, while total leukocyte counts remained fairly constant (not shown). Icterus index values which were determined on three dogs are shown to have increased by as much as four-fold (at the top of fig. 1). The cessation of fat feeding in two dogs resulted in rapid return of red cell counts to normal (long dashes, fig. 1), while the discontinuation of both fat and choline feeding (short dashes) also was followed by returns of the erythrocyte numbers to normal in the other two dogs.

The administration of 60 grams of lard to three dogs caused no significant, uniform changes in their erythrocyte counts; while the administration of lard to five dogs which had been made mildly anemic by daily choline-feeding for three weeks or more, simply caused fluctuations in the cell counts. Since these

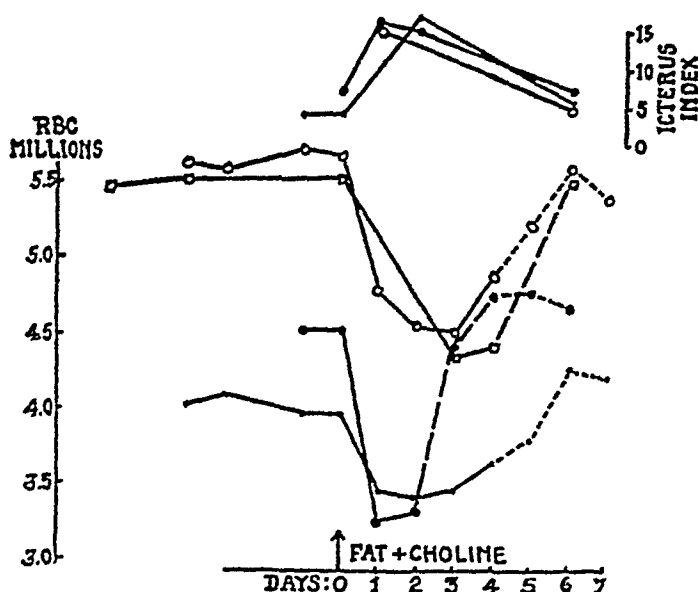


Fig. 1. Effect of lard and choline feeding on the erythrocyte numbers of four normal dogs

Long dashes indicate cessation of fat feeding. Short dashes indicate cessation of both fat and choline administration. Icterus index values on three dogs are given at top of the figure.

last-mentioned findings do not contribute positive results, they are not shown graphically.

DISCUSSION. The fact that erythrocyte numbers and hemoglobin were lowered rapidly in these experiments (fig. 1) while the icterus indices were raised indicates that a hemolytic action is involved. In as much as the discontinuation of fat feeding allowed the cell counts and icterus indices to return to normal, we believe that the fat feeding is responsible for the increased rate of cell destruction reflected by the decrease in erythrocyte number. Choline feeding alone does not cause a significant fall in erythrocyte numbers until it has been administered daily for 8 or more days (5, 4). Yet the simultaneous administration of choline is necessary for fat feeding to lower the red cell count uniformly and significantly in these experiments. We believe that choline acts as previously

postulated (6) by causing vasodilatation and improved blood and oxygen supply to the bone marrow and thus depressing erythropoiesis. Although choline alone cannot lower the erythrocyte count within 24 hours it may act as a weak brake which inhibits any *acceleration of erythropoiesis* which may normally follow the hemolytic destruction of red cells.

The hemolytic products of fat digestion which escape resynthesis to fat during absorption (4) would be able to lower the red blood cell count if such a "brake" (as choline may be) were to prevent compensatory erythropoiesis from keeping pace with hemolysis. Indeed, Dupee et al. (7) have shown that a high fat diet causes bone marrow hyperplasia in dogs.

We realize that there may be other theoretical explanations of the rôle of choline in these experiments, but the postulated mechanism of action given above seems most feasible to this author. In previous work (6) it appeared that choline depressed erythropoiesis by a vascular action, since atropine blocked its effect.

We are aware of the fact that two of our dogs had initial red blood cell counts which were too low to be considered normal. They had been recently received by the laboratory and, in the absence of more acclimatized dogs, were used for this experiment. We do not believe that the initial low red cell counts of the two dogs had any significant effect upon the general results obtained.

CONCLUSIONS

The daily oral administration of 60 grams of lard and 10 mgm. per kgm. of choline hydrochloride to four normal dogs caused rapid, significant reductions in their erythrocyte counts and hemoglobin percentages. In three of the dogs, the erythrocyte numbers were observed to be diminished by 15 to 27 per cent within the first 24 hours. Icterus indices were significantly elevated concomitantly.

Discontinuation of fat feeding alone, or of both fat and choline administration resulted in rapid returns of erythrocyte numbers to normal.

These results are interpreted by assuming that the choline acts as a brake on the bone marrow in preventing any great acceleration of erythropoiesis, while the fat furnishes hemolytic agents (perhaps soaps and fatty acids) which increase red cell destruction.

REFERENCES

- (1) LONGINI AND JOHNSON. This Journal 140: 349, 1943.
- (2) LOEWY, FREEMAN, MARCHELLO AND JOHNSON. This Journal 138: 230, 1943.
- (3) JOHNSON, LONGINI AND FREEMAN. Science 97: 400, 1943.
- (4) FREEMAN AND JOHNSON. This Journal 130: 723, 1940.
- (5) DAVIS. This Journal 127: 322, 1939.
- (6) DAVIS. This Journal (in press).
- (7) DUPEE, JOHNSON, MARCHELLO, WILNER AND FREEMAN. Fed. Proc. 3: 8, 1944.

STIMULATION OF DENERVATED SKELETAL MUSCLE WITH ALTERNATING CURRENT¹

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The response of normal nerve to sinusoidal alternating current has been studied by a number of investigators (1, 2, 3, 4, 5, 6). A number of quantitative treatments of the excitation data have appeared, for example that of A. V. Hill and his co-workers (5). This form of current possesses certain characteristics which might be of value in the application of electrical stimulation to the treatment of denervated muscle (7). The present study was undertaken to obtain information regarding the responses of denervated skeletal muscle to alternating current stimulation with special reference to its application in clinical electrotherapy.

METHODS. The gastrocnemius-soleus muscles of 5 dogs were denervated on one side by section of the sciatic nerve high in the thigh. A large segment of nerve was resected to guard against regeneration. At weekly intervals following denervation, the animals were anesthetized with nembutal and the responses of the gastrocnemius muscle to alternating current stimulation were studied. Since we were primarily interested in clinical application rather than in the quantitative verification of any particular theory of excitation, stimulation was performed as it would be done clinically. Percutaneous stimulation using usually the monopolar technique was employed. A large dispersive electrode was placed upon the shaved abdomen and a small (1.5 cm.) stimulating electrode was held against the belly of the gastrocnemius. In some instances rats were used. In these animals, the gastrocnemius was freed from the surrounding muscles leaving its blood supply intact. The tendon was attached to an optically recording isometric lever; the femur was held in a rigid clamp. Stimulation was accomplished through two steel needle electrodes, one through the muscle belly and the other through the tendon.

The apparatus employed for stimulation was a specially designed electronic generator which has been described in detail elsewhere (7, 8). Intensity readings always refer to "steady stimulation".

RESULTS. 1. *Frequency-intensity relation for minimal stimulation.* Figure 1 shows a typical frequency-intensity curve for threshold stimulation. The curve for the 28 day denervated muscle is compared with a normal control curve taken on the same muscle 3 days preoperatively. Both curves are U-shaped with a more or less definite optimum frequency. For normal muscle, the latter was found to be usually between 60 and 100 cycles per second under the experimental conditions employed in this study. For the 28 day denervated muscle, the optimum was found to be much lower and is approximately 0.75 cycle per second. Another point of interest is the tendency of the normal curve to flatten

¹ Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

out in the extremely low frequency range. This is undoubtedly related to the "breakdown of accommodation" described by Skoglund (9).

Figure 2 shows the response of the same muscle 40 days after denervation. This curve is flatter on the low side of the optimum frequency than the 28 day curve.

2. *The influence of the index of response upon the shape of the intensity-frequency curves.* Figure 3 shows the shape of the frequency-intensity curves for a 42 day denervated muscle when three different indices of response were employed. The lowest curve represents threshold stimulation of the unloaded muscle. An optimum frequency exists at about 3 cycles per second and the curve is relatively flat below the optimum frequency. The middle curve represents a maximal response in the same muscle lifting a 500 gram weight. By changing the index of response, the entire picture is altered. The optimum frequency is now 25 cycles per second and the curve rises steeply at frequencies below 10 cycles. In the upper curve, the muscle was made to lift a weight of 1000 grams. In this

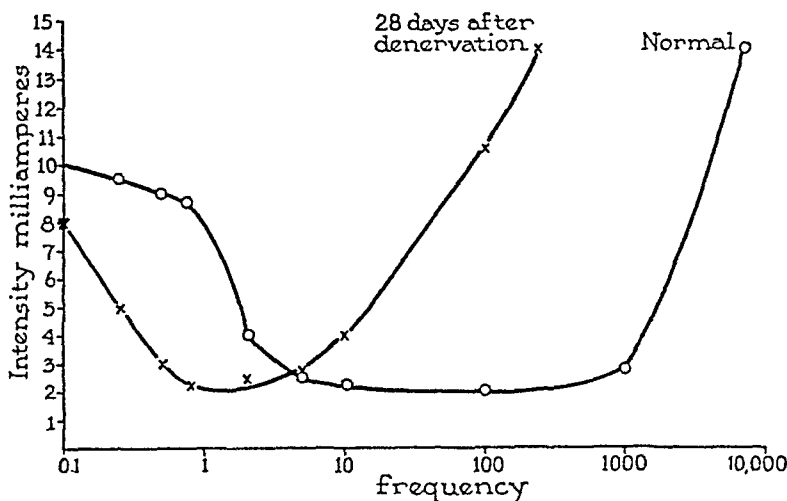


Fig. 1. Intensity-frequency curves for normal and 28-day denervated gastrocnemius muscle of the dog.

case the optimum frequency is again 25 cycles but the curve rises even more steeply below 10 cycles.

Figure 4 represents a family of curves in which the relative current strengths for different indices of response are compared for several different frequencies. A 25 cycle current required about twice the intensity when index 2 was used as compared to index 1. For index 3, about 3.5 times the intensity was required. As the frequency is lowered, the slope of the curves becomes much steeper so that at 0.25 cycle, over 14 times as much current is required for index 2 compared to index 1.

In figure 5, the relative current intensities required at different frequencies are compared to the intensity required at 25 cycles for different indices of response. At the higher indices this ratio increases markedly. In fact, in this particular experiment the 3rd index could not be attained with frequencies of 0.08 and 0.25 cycles at 130 ma. which was the limit of intensity obtainable with our apparatus.

The curves shown in figures 3, 4 and 5 are typical of all the results obtained in the 5 animals which were studied. Similar results were obtained in rats.

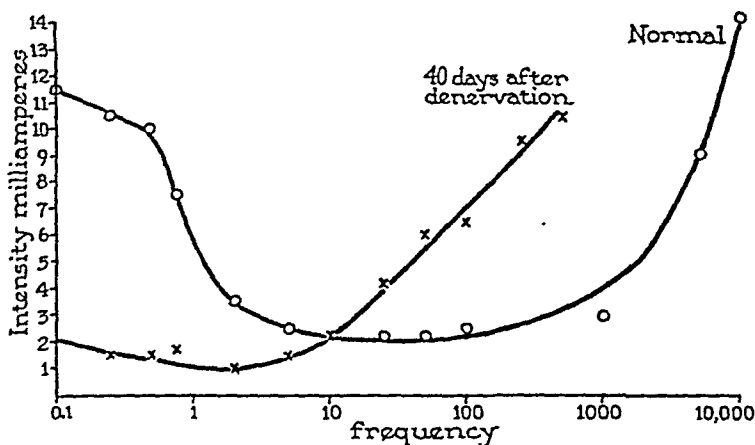


Fig. 2

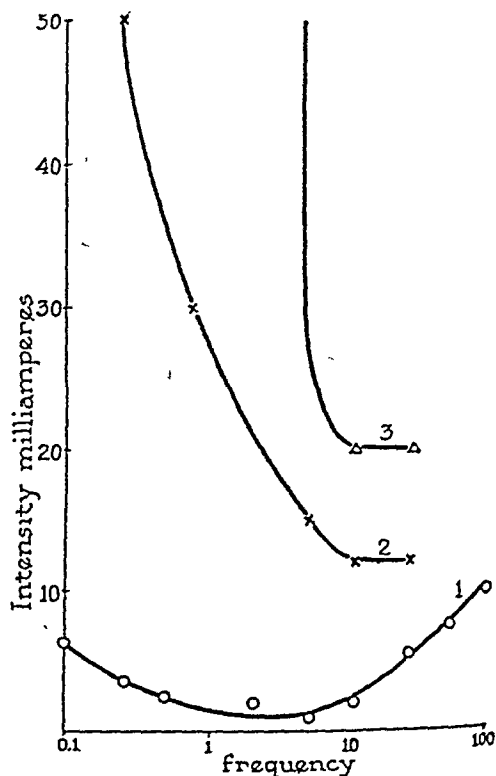


Fig. 3

Fig. 2. Intensity-frequency curves for normal and 40-day denervated gastrocnemius muscle of the dog.

Fig. 3. Intensity-frequency relation in denervated dog gastrocnemius for different indices of response. Curve 1 = Minimal threshold (Index 1). Curve 2 = Maximal contraction with 500 gram load (Index 2). Curve 3 = Maximal contraction with 1000 gram load (Index 3).

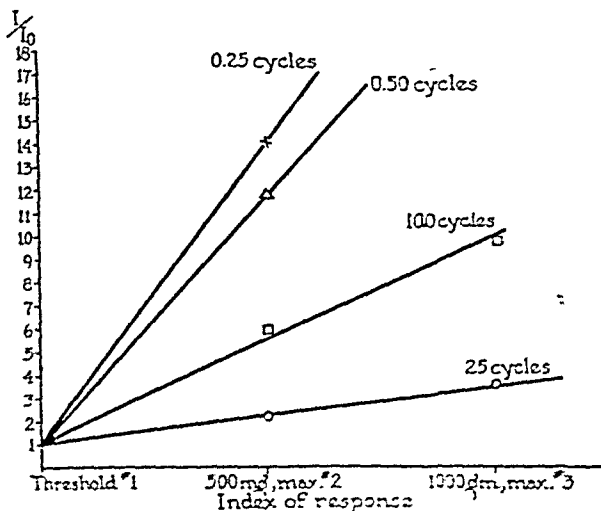


Fig. 4

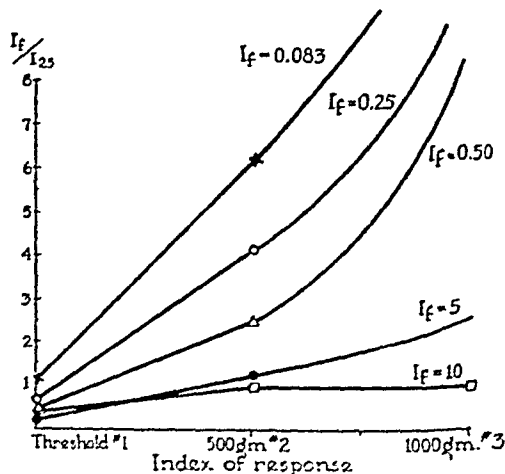


Fig. 5

Fig. 4. Relative current strengths for different indices of response in denervated dog muscle.

Fig. 5. Current strengths for different indices of response relative to that for a 25 cycle current.

DISCUSSION. We are particularly interested in the application of these results to the interpretation of the mechanical response in denervated muscle and to the therapeutic use of electrical muscle stimulation in the clinic. We are not concerned at this time with the quantitative mathematical treatments of Hill and others (6), although certain general principles involved in these theories will be referred to.

It has been stressed (10, 11) that electrical muscle stimulation, in order to be of value in delaying atrophy in denervated skeletal muscle, must produce vigorous contractions. It has also been stated that currents of sufficient intensity to produce vigorous contractions could never be employed clinically (11, 12). We have suggested (10) that a stimulus which produces a tetanic contraction which closely simulates normal voluntary effort might be the best for electrical stimulation of denervated muscle, and we have shown that this type of current at a relatively low intensity delays atrophy in the denervated gastrocnemius of the rat (7). Recently, the mechanism of the "slow contraction" of denervated muscle has received renewed attention (12). The results of the present study have a bearing on all of these phenomena.

Bremer (13) thought that the slow reponse of denervated muscle was due to a "neuromuscular contracture". More recent evidence, however, is against this interpretation. For example, Rosenblueth and Luco (14) and Doupe (12) have shown that the slow response is accompanied by conducted action potentials and hence is not a true contracture. Moreover, both Bremer and Doupe have shown that the slow response is obtained with currents of long duration or slow rate of rise and that it may be absent when brief, rapidly rising stimuli are employed. It has been known for a long time (V. Kries, 1884 (15); Hoffman, 1910 (16)) that slowly rising stimuli lead to repetitive discharges in normal amphibian muscle which greatly modify the size and form of the muscle contraction. More recently, the same phenomenon has been shown to occur in normal mammalian muscle (Schriever and Cebulla, 1938 (17); Granit and Skoglund, 1941 (18); Skoglund, 1942 (9)). In terms of Hill's theory, one might reasonably expect repetitiousness to occur readily in denervated muscle because the latter is a tissue of slow accommodation. In view of these considerations, Doupe suggested that the slow response in denervated muscle was due to repetitiousness and that a constant or slowly rising stimulus produced what amounted to a tetanic contraction.

Against this concept is Bremer's demonstration that the tension developed by the slow component never amounted to more than one-fifth of that of a maximal twitch. This meant that if a tetanus were responsible for the slow component, it involved only a small proportion of the muscle fibers or the individual fibers were contracting asynchronously at slow rates, or that there was some fundamental difference in the tension developed in response to slow and rapid stimuli.

From the practical point of view, we are concerned with the mechanism of the response of denervated muscle to different forms of stimuli and its ability to develop tension. For example, clinical physical-therapists rely chiefly upon the so-called "slow sinusoidal" currents for the stimulation of denervated muscle.

(This is the only current they now have available on their generators which remotely approximates an appropriate stimulus for denervated muscle.) The question arises whether there would be any advantage in substituting a 25 cycle current inasmuch as the slow sinusoidal type may produce a true tetanus as a result of repetitiousness. The results of this study give an answer to this question.

It is clear that the ability to develop tension is much greater when a frequency of 25 cycles per second is employed as compared to the "slow sinusoidal" type. In other words the contraction elicited by the 25 cycle stimulus can perform considerable work whereas the slow contraction elicited by the "slow sinusoidal" stimulus cannot. This means that if the slow sustained response obtained with the "slow sinusoidal" stimulus is tetanic in nature, it must differ in some way from that initiated by a 25 cycle stimulus.

[We believe that the slow response in denervated muscle is due to repetitive responses as suggested by Doupe (12). A clue to the difference in the nature of the tetanic response to slow sinusoidal current and that to a 25 cycle current is found in the work of Skoglund (9). This author found that when action currents were recorded from a group of mammalian motor nerve fibers, small spike potentials were easily obtained with slowly rising currents of moderate intensity but in order to obtain large spike potentials, much greater current intensities were required with slowly rising stimuli. In other words, the accommodation curve constructed when large spikes were used as an index were much steeper than when small spikes were selected as the index of response. This finding is exactly analogous to our results with alternating current. As the index of response was increased, the slope of the curve on the low side of the optimum frequency became much more steep corresponding to the steeper accommodation curve noted by Skoglund. The threshold for grouped or synchronized responses appears to be very high when slowly rising stimuli are employed.

What are the practical implications of this phenomenon in relation to electrotherapy? First it should be realized that the fundamental nature of the muscle response to different forms of stimuli may be very different and that the nature of the response may have significance in regard to the success or failure of electrotherapy. Secondly, if it is true that in order to accomplish satisfactory results with electrical muscle stimulation strong vigorous contractions must be produced, the type of stimulus to be used becomes very important. For example, a 25 cycle current will usually produce a maximal response in a denervated muscle loaded with 1000 grams at an intensity of only 10 to 15 ma. This intensity is very easily tolerated. On the other hand, a "slow sinusoidal" stimulus of over 125 ma. was often unable to produce one quarter of a maximal response in the same muscle. Hence the statements (10, 12) that current intensities sufficient to produce vigorous contractions could never be used clinically does not appear to be true if care is exercised in selecting the current for stimulation.

SUMMARY AND CONCLUSIONS

1. For threshold stimulation with alternating current, denervated muscle has an optimum frequency in the neighborhood of 1 to 3 cycles per second.

2. The optimum frequency and the slope of the intensity-frequency curve is a function of the index of response chosen to evaluate it. When the muscle is called upon to do considerable work or exert considerable tension, the optimum frequency is about 25 cycles per second and the slope of the curve below this point becomes very steep.

3. The relation of these results to the interpretation of the mechanical response in denervated muscle and to practical electrotherapy is discussed.

REFERENCES

- (1) VON KRIES, J. Ber. Verh. naturf. Ges., Freiburg **8**: 170, 1884.
- (2) ACHELIS, J. D. Pflüger's Arch. **224**: 217, 1930.
- (3) LULLIES, H. Pflüger's Arch. **225**: 98, 1930.
- (4) COPPÉE, G. Arch. int. Physiol. **40**: 1, 1934.
- (5) COPPÉE, G. Cold Spring Harbor Symp. Quant. Biol. **4**: 150, 1936.
- (6) HILL, A. V., B. KATZ AND D. Y. SOLANDT. Proc. Roy. Soc. London **B121**: 74, 1936.
- (7) OSBORNE, S. L., F. S. GRODINS, E. MITTELMANN, W. S. MILNE AND A. C. IVY. Arch. Physical Therap. **25**: 338, 1944.
- (8) MITTELMANN, E., F. S. GRODINS AND A. C. IVY. Electronics **16**: 132, 1943.
- (9) SKOGLUND, C. R. Acta Physiol. Scandinav. **4**: (Suppl. XII), 1942.
- (10) GRODINS, F. S., S. L. OSBORNE AND A. C. IVY. Arch. Phys. Therap. **23**: 729, 1942.
- (11) HINES, H. M., J. D. THOMSON AND B. LAZERE. Arch. Phys. Therap. **24**: 69, 1943.
- (12) DOUPE, J. J. Neurol. and Psychiatry **6**: 136, 141, 1943.
- (13) BREMER, F. J. Physiol. **76**: 65, 1932.
- (14) ROSENBLUETH, A. AND J. V. LUCCO. This Journal **120**: 781, 1937.
- (15) VON KRIES, J. Arch. Anat. Physiol. **8**: 337, 1884.
- (16) HOFFMANN, P. Arch. Anat. Physiol. **34**: 247, 1910.
- (17) SCHRIEVER, H. AND R. CEBULLA. Pflüger's Arch. **241**: 1, 1938.
- (18) GRANIT, R. AND C. R. SKOGLUND. Nordisk, Medicinsk Tidskrift **13**: 138, 1942.

THE EFFECT OF APPROPRIATE ELECTRICAL STIMULATION ON ATROPHY OF DENERVATED SKELETAL MUSCLE IN THE RAT¹

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The literature is not in agreement regarding the effect of the electrical stimulation of denervated skeletal muscle on its rate of loss of weight and strength (1). Some investigators have observed that the rate is retarded (2-5) and others have observed either no effect or an unfavorable effect (6-8). This study was undertaken with the idea *a*, that the most appropriate electrical stimulus for degenerating muscle has not been determined and applied, and *b*, that a type of current could be found which would produce a smooth tetanic contraction of denervated muscle without causing normal muscle to contract and without causing pain.

The *electrical stimulating apparatus* used in this study has been described in detail elsewhere (9). It is a wide frequency range electronic generator with a frequency range of from 0 to 150,000 cycles per second. With it many wave forms of varying intensity may be generated.

METHODS. Adult albino rats were used. They were kept in individual cages with a thick layer of shavings on the bottoms. In spite of every reasonable precaution, many of the denervated animals developed severe trophic ulcers on their feet and had to be discarded. These animals showed a marked tendency toward eating their own feet, a difficulty which has been encountered by other workers. The sciatic nerves were sectioned bilaterally under ether anesthesia. A large segment of the nerve was resected to guard against regeneration. The gastrocnemius muscle of one side was given daily electrical stimulation and the other side served as a control.

Stimulation was carried out under light ether anesthesia, the leg being held in a special clamp and the foot loaded with a 20 gram weight. A small (0.5 cm.) stimulating electrode was held against the belly of the gastrocnemius and a large dispersive electrode was placed on the abdomen. The current intensity was adjusted to yield maximal contractions. Different frequencies were used.

At the end of a specified period, the animals were sacrificed, the gastrocnemius muscles dissected out and immediately weighed. In all instances the nerves were traced to make certain that continuity had not been re-established. Twenty-four hours were allowed to elapse between the last period of stimulation and the sacrifice of the animals in order to avoid errors due to any transient change in muscle weight which might result from changes in blood flow or water content during stimulation.

RESULTS. 1. *Control series.* To permit an accurate evaluation of the results of electrical stimulation, it is first necessary to know *a*, the difference in the weight of the normal gastrocnemius on the right and left sides of the same

¹ Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

(normal or intact) animal, and *b*, the difference in the rate of loss of weight on the right and left sides of the same animal following bilateral sciatic section in the absence of electrical stimulation. The results of these control studies are given in tables 1 and 2. It will be seen that the right and left gastrocnemius muscles of the same animal may differ in weight by 0.4 to 4.7 per cent with an average difference of 1.76 per cent. The difference in weight favored the right side with about the same frequency as the left. The average weights of the right and left sides for the entire group of animals were coincidentally exactly equal. From table 2 it will be seen that the maximum difference in weight between corresponding pairs of untreated denervated muscles was 5.7 per cent and the average difference about 2 per cent.

TABLE 1

Normal rats to show difference in weight of right and left gastrocnemii and relation of normal muscle weight to normal body weight

BODY WEIGHT	WT. GASTROCNEMIUS		% DIFF.	AVE. WT.	(MUSCLE WT./BODY WT.) $\times 100$		
	Right	Left			Right	Left	Ave.
196	1.110	1.105	0.5	1.108	0.566	0.564	0.565
239	1.390	1.370	1.5	1.380	0.580	0.571	0.577
202	1.165	1.170	0.4	1.675	0.576	0.580	0.578
185	1.000	1.050	5.0	1.025	0.541	0.561	0.554
190	1.130	1.150	1.8	1.140	0.594	0.605	0.600
200	1.140	1.110	2.6	1.125	0.570	0.555	0.562
218	1.175	1.185	0.8	1.180	0.540	0.544	0.541
213	1.225	1.170	4.7	1.198	0.575	0.550	0.562
284	1.630	1.650	1.2	1.640	0.574	0.581	0.577
240	1.330	1.375	3.4	1.352	0.554	0.573	0.563
256	1.665	1.635	1.8	1.650	0.650	0.639	0.644
Ave. 220	1.269	1.269	$\begin{cases} 1.76 \\ 0.00 \end{cases}$	1.269	0.575	0.575	0.575

Left side heavier in 6 of 11 or 54.5 per cent.*

Right side heavier in 5 of 11 or 45.5 per cent.

By coincidence the average weights were identical.

Comment. These results provide a basis for the evaluation of electrical stimulation. We should be justified in attributing any difference in weight of 1 per cent or more to the effects of the treatment employed. These control figures agree well with the findings of Langley and Hashimoto (10) in the rabbit. They found that 75 per cent of normal muscle pairs differed in weight by less than 4 per cent and in 92 per cent of cases by less than 6 per cent. Three weeks after denervation, 73 per cent of corresponding muscle pairs differed in weight by less than 6 per cent.

Figure 1 shows the average rate of weight loss in the untreated denervated gastrocnemius muscle of the rat. The experimental curve corresponds closely to the equation of Knowlton and Hines (11):

$$k = \frac{1}{D - 1.5} \log \frac{85.5}{85.5 - X}$$

TABLE 2

Denervated rats to show the difference in the rate of weight loss on the two sides of the same animal

DAYS DENERVATED	GASTROCNEMIUS wt.				(MUSCLE WT./BODY WT.) $\times 100$			
	Body wt.	Right	Left	% diff.	Right	Left	Ave.	% of normal
	<i>gms.</i>							
5	212	1.175	1.195	1.7	0.565	0.574		
5	283	1.425	1.435	0.7	0.505	0.509		
5	258	1.255	1.290	2.8	0.487	0.500		
5	215	1.070	1.020	4.9	0.498	0.475		
Mean....	242	1.230	1.232	2.5 (0.2)	0.514	0.514	0.514	89.4
10	190	0.660	0.665	0.8	0.348	0.350		
10	202	0.870	0.850	2.3	0.439	0.429		
10	218	0.940	0.930	1.1	0.431	0.426		
10	176	0.755	0.755	0.0	0.429	0.429		
Mean.....	196	0.806	0.800	1.0 (0.7)	0.412	0.408	0.410	71.3
15	192	0.530	0.540	1.9	0.276	0.282		
15	196	0.680	0.690	1.5	0.347	0.352		
15	208	0.650	0.650	0.0	0.313	0.313		
15	228	0.705	0.750	5.7	0.309	0.328		
Mean	206	0.641	0.657	2.3 (2.5)	0.311	0.319	0.315	54.8
20	227	0.585	0.600	2.6	0.258	0.264		
20	236	0.625	0.625	0.0	0.265	0.265		
20	252	0.720	0.730	1.4	0.284	0.290		
20	231	0.605	0.625	3.3	0.262	0.271		
20	258	0.730	0.730	0.0	0.283	0.283		
Mean....	241	0.653	0.662	1.5 (1.4)	0.270	0.275	0.272	47.3
25	256	0.550	0.535	2.6	0.207	0.201		
25	226	0.530	0.515	2.9	0.234	0.228		
25	238	0.565	0.560	0.9	0.237	0.240		
Mean....	240	0.518	0.537	2.0 (2.0)	0.226	0.223	0.224	39.0
30	276	0.610	0.622	2.0	0.221	0.225		
30	282	0.525	0.570	3.8	0.186	0.202		
30	358	0.740	0.740	0.0	0.206	0.206		
30	330	0.640	0.680	6.2	0.194	0.206		
Mean...	312	0.629	0.653	3.0 (3.8)	0.202	0.209	0.205	35.7

Where D represents the number of days after denervation and X the percentage of weight loss. For our colony, k was equal to 0.020 which is in fair agreement with the value of 0.029 obtained by Knowlton and Hines (11).

2. *Treated series.* Several series of animals have been treated with electrical stimulation. The results are summarized in tables 3 to 6.

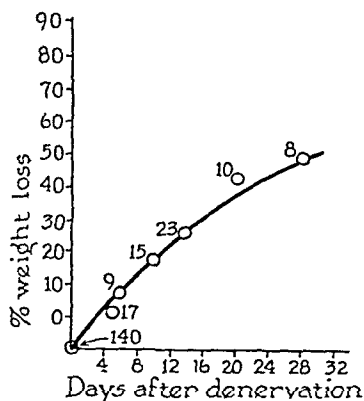


Fig. 1

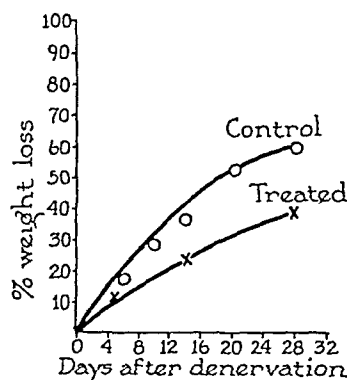


Fig. 3

Fig. 1. Rate of atrophy of the gastrocnemius muscle of the rat following denervation. Each experimental point is the average of the number of observations indicated.

Fig. 3. Comparison of the rate of atrophy in control muscles and in muscles stimulated for 15 minutes daily with 25 cycle alternating current.

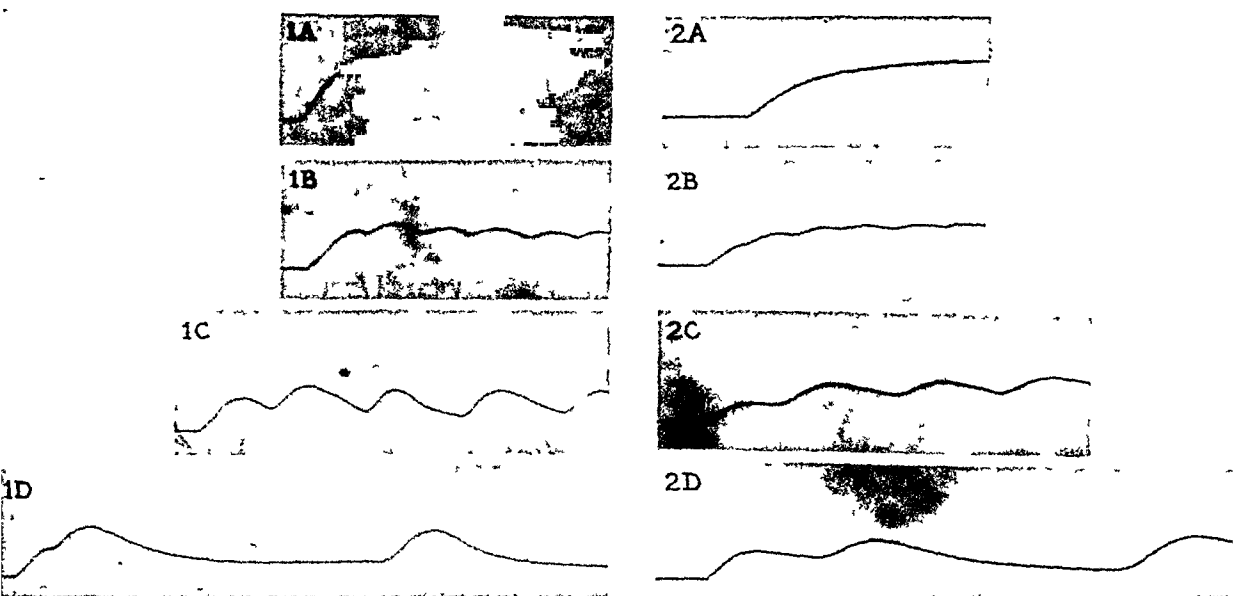


Fig. 2. Optically recorded isometric myograms showing the genesis of tetanus in normal and denervated gastrocnemius of the rat stimulated with sinusoidal alternating current of different frequencies.

1A—Normal; 25 cycles. 1B—Normal; 10 cycles. 1C—Normal; 5 cycles. 1D—Normal; 2 cycles. 2A—Denervated; 25 cycles. 2B—Denervated; 10 cycles. 2C—Denervated; 5 cycles. 2D—Denervated; 2 cycles.

Group A—25 cycle alternating current. Some of the theoretical reasons for the use of a low frequency sinusoidal current have been considered elsewhere (1, 12). A frequency of 25 cycles was chosen for study because it was found in preliminary experiments that this frequency, when used to stimulate *denervated* rat muscle, produced a, a completely fused smooth tetanic contraction as shown in figure 2;

TABLE 3
Unilateral stimulation with 25 cycle AC for 10 minutes daily

RAT NO.	DAY SACRIFICED	BODY WT.		GASTROCNEMIUS WT.		As % OF BODY WT.		% DIFF.
		Initial	Final	Left	Right	Left	Right	
8	9	316	278	1.26	1.33*	0.45	0.48	+5.6
4	11	394	362	1.68*	1.26	0.46	0.35	+33.3
7	11	410	371	1.85	1.91*	0.50	0.51	+3.4
1	14	317	260	1.11*	0.91	0.41	0.35	+22.0
5	14	278	244	1.00*	0.98	0.41	0.40	+2.0
6	14	268	243	0.93*	0.85	0.38	0.36	+9.4
9	14	270	253	1.00*	0.85	0.38	0.34	+17.7
10	14	208	191	0.88*	0.62	0.46	0.32	+42.0

* Treated muscle.

Summary

NO. DAYS	NO. RATS	AVE. % WT. DIFF.
9	1	+5.6
11	2	+18.6
14	5	+18.6

TABLE 4
Unilateral stimulation with 25 cycle A.C. for 15 minutes daily
(14 days)

RAT NO.	DAY SACRIFICED	BODY WT.		GASTROCNEMIUS WT.		As % OF B. W.		% DIFF.
		Initial	Final	Left	Right	Left	Right	
1	14	274	244	0.91	1.01*	0.37	0.41	+11.0
2	14	255	225	0.85	1.12*	0.38	0.50	+31.8
3	14	321	302	1.035	1.225*	0.34	0.41	+18.3
4	14	232	227	1.130*	0.810	0.50	0.36	+39.5
5	14	306	270	1.165*	1.040	0.43	0.38	+12.0
6	14	356	310	1.250*	1.100	0.40	0.35	+13.6
7	14	232	188	0.930*	0.760	0.50	0.40	+22.4
9	14	356	310	1.250*	1.100	0.40	0.35	+13.6
10	14	318	280	1.280*	1.200	0.46	0.43	+6.7

Summary

DAYS	NO. RATS	AVE. % DIFF. WT.
14	9	+19.6

* Treated muscle.

b, a maximal isometric tension in comparison with currents of lower or higher frequencies; *c*, comparatively little or no stimulation of neighboring normal muscle at the required intensities. The current intensity required to produce

maximal contractions was 1.5 to 2.0 ma. The carrier frequency was modulated by a "surging" mechanism to produce 40 contractions per minute.

Table 3 shows the results of stimulation for a *period of 10 minutes* daily. In each individual animal the treated muscle was the heavier. If we consider any

TABLE 5
Unilateral stimulation with 25 cycle A.C. for 15 minutes daily
(28 days)

RAT NO.	BODY WEIGHT		GASTROCNEMIUS WT.		As % B. W.		% DIFF.
	Initial	Final	Left	Right	Left	Right	
1	227	204	0.530	0.795*	0.26	0.39	+50.0
2	344	300	0.575	0.855*	0.19	0.29	+48.8
3	234	196	0.765*	0.525	0.39	0.27	+45.7
4	226	214	0.900*	0.465	0.42	0.22	+93.5
5	296	255	1.005*	0.660	0.39	0.26	+52.4
6	266	242	0.530	0.715*	0.22	0.30	+35.0
7	190	172	0.540*	0.370	0.31	0.22	+46.0
8	182	200	0.530*	0.360	0.26	0.18	+47.2

Summary

NO. DAYS	NO. RATS	AVE. % WT. DIFF.
28	8	+52.5

* Treated muscle.

TABLE 6
Unilateral stimulation with "slow sinusoidal" current for 15 minutes daily

RAT NO.	DAY SACRIFICED	BODY WEIGHT		GASTROCNEMIUS WT.		As % B. W.		% DIFF.
		Initial	Final	Left	Right	Left	Right	
1	14	390	358	1.26	1.38*	0.35	0.39	+9.5
2	14	230	214	0.81	0.86*	0.38	0.40	+6.2
3	14	352	346	1.42*	1.27	0.41	0.38	+11.8
4	14	192	174	0.50*	0.61	0.29	0.35	-22.0
5	14	400	338	1.27	1.33*	0.38	0.39	+4.7
6	14	230	216	0.95	0.80*	0.44	0.37	-18.7
7	14	378	329	1.32*	1.35	0.40	0.41	-2.3
8	14	356	334	1.35	1.48*	0.40	0.44	+9.6
9	14	310	284	1.12	1.12*	0.39	0.39	0.0

* Treated muscle.

difference over 10 per cent to be significant, then the treatment produced a significant increase in weight in 3 of 5 cases after a period of 14 days.

Tables 4 and 5 show the results obtained with a 15 minute period of stimulation. After 14 days (table 4) the stimulated side was heavier in every case and a significant increase in weight was produced in 8 of 9 animals.

After 28 days (table 5) the differences were very striking. A markedly significant increase in weight was produced in all eight animals. The increases ranged from 35 per cent to 93 per cent with an average difference of 52.5 per cent.

Figure 3 compares the average rate of weight loss of the stimulated (15 min. period) and control muscles. In constructing this curve, muscle weights were expressed in terms of percentage of body weight and compared with the normal gastrocnemius-body weight ratio of 0.575 per cent. The latter figure represents the average of 70 normal rats or 140 separate muscles. These values ranged from 0.502 to 0.640 with a standard error of the mean of 0.004.²

The curve of atrophy for the treated muscles can be fitted to the same general equation as given above. In this case, k has a value of 0.010, compared to 0.020 for the untreated muscles.

Group B—"Slow sinusoidal" current as supplied by generators now used clinically. Table 6 summarizes the results obtained with a "slow sinusoidal" current of the type supplied by most of the generators available for clinical use. A 15 minute period of stimulation was used and 6 to 7 ma. of current were usually required. Contractions were produced at the rate of 40 per minute. With this current, the results obtained were much more variable. Of the nine animals in the group, the treated side was significantly heavier in only one instance, the control muscle was heavier in two cases, and in the remaining six animals there was no significant difference in weight between the control and treated muscles.

DISCUSSION. The results obtained in this study show that stimulation with a 25 cycle alternating current for a period of 10 or 15 minutes daily is markedly effective in retarding atrophy in the denervated gastrocnemius of the rat. This result is in agreement with the findings of Fischer (2), Solandt (4) and Hines (5) in the rat, and of Gutmann and Guttman (3) in the rabbit. The difference in weight between the treated and control muscles becomes much more marked as time elapses. Thus an average difference of 19.6 per cent after 14 days increased to a difference of 52.5 per cent after 28 days. If the logarithmic curves of atrophy which fit the data for the first 28 days continue to be followed, we would expect a difference of some 80 per cent in the weights treated and control muscles after 60 days.

We were unable to demonstrate a beneficial effect when a so-called "slow sinusoidal" current, as supplied by most generators now available for clinical use, was employed for a period of 14 days. In this we are in agreement with Solandt (4) who reported that 25 cycle sinusoidal current was more effective than galvanic current. At present, we have a series of animals under treatment with interrupted galvanic current. The series is not yet complete but in 5 animals which have been followed for 28 days, the treated muscles averaged 12.5 per cent heavier than their controls. These results indicate that this type of current may be of some benefit but is inferior to the 25 cycle current.

It thus appears that the type of current to be used in stimulating denervated muscle is not a matter of indifference. The question arises as to why such a difference should exist. From certain theoretical considerations (1,12), we were

² These values are for the gastrocnemius alone,—the soleus is not included.

led to the conclusion that low frequency sinusoidal alternating current might be best for the stimulation of denervated muscle.

Our experiments on the genesis of tetanus and the tension-frequency relationship in denervated rat muscle pointed to a frequency of about 25 cycles as the optimum. The type of response produced in denervated rat muscle when a 25 cycle current was employed differed fundamentally from that produced by "slow sinusoidal" or interrupted galvanic currents. In the former case a smoothly graded tetanic response was obtained which closely stimulated a normal voluntary effort. In the latter instances, the contractions were brief twitches. These twitches were very brisk for the first 5 to 7 days following denervation after which they became somewhat more sluggish, particularly with the "slow sinusoidal" current. When the frequency of this "slow sinusoidal" current was lowered so that the individual pulses rose very slowly, this sluggishness was much more marked.

The nature of this slow response in denervated muscle does not appear to be well understood at the present time (13-17). Bremer, however, has found that the tension developed by the slow component never amounted to more than one-fifth of that of the twitch. This means that if the slow component were tetanic in nature, either *a*, it must involve only a small proportion of the muscle fibers, or *b*, the individual fibers are responding asynchronously at relatively slow rates, or *c*, the tension developed by repetitive responses to a constant stimulus differs from that developed by repetitive responses to repeated external stimuli. Whatever the actual mechanism of this slow response may be, it would appear that the response of a denervated muscle to 25 cycle current more closely simulates a normal voluntary contraction than those produced by the direct (galvanic) or "slow sinusoidal" stimuli.

Solandt (4) has suggested that 25 cycle alternating current was close to the optimum frequency for stimulation of denervated muscle. We (18) have found the optimum frequency for *threshold* stimulation to be in the neighborhood of 2 to 5 cycles. However, the maximum possible isometric tension was developed at 25 cycles. Experiments in our laboratory in which denervated muscle was stimulated with sinusoidal alternating current of different frequencies (0.083-500 cycles per sec.) have shown that the optimum frequency is a function of the index of response which is chosen. This means that the optimum frequency and the slope of the accommodation curve are functions of the index chosen to evaluate them. Solandt has also suggested that fatigue developed less rapidly at 25 cycles, as compared with one of 60 cycles. We have found this to be true in the rat and Rosenblueth and Luco (17) reported a similar finding in the cat.

One other important point remains to be considered. It has been stressed that vigorous exercise is apparently necessary to obtain significant benefit from electrical stimulation (1,5,8). It has been suggested (5,8) that the current intensities necessary to produce contractions of sufficient vigor to be beneficial could never be employed on a patient. This implies that the beneficial results shown in animal experimentation can have no practical value for the clinician. If care is exercised in the selection of a current for stimulation, this may not be

true. For example, a markedly beneficial effect was produced in the rat with a 25 cycle current of only 2 ma. In several cases of facial paralysis, we have obtained vigorous responses with 8 to 12 ma., an intensity which is easily tolerated.

In a further attempt to simulate actual clinical conditions as closely as possible and still retain the control which is possible only in animal experiments, we have trained a group of dogs to allow stimulation *without anesthesia or sedation* of any kind. In this way, the practical limitations of tolerance are not likely to be exceeded. In these animals, we have been able to produce maximal tetanic contractions in the denervated gastrocnemius muscle working against a load of 500 grams with a 25 cycle sinusoidal current of only 6 to 7 ma. This is still true 3 months after denervation. The current is tolerated easily; in fact, many of the animals go to sleep during the treatment period.

SUMMARY AND CONCLUSIONS

1. Stimulation with 25 cycle alternating current markedly diminishes the rate of atrophy in the denervated gastrocnemius muscle of the rat.

2. No such beneficial effect was demonstrated with a "slow sinusoidal" current.

3. Some theoretical and practical aspects of electrical stimulation are discussed.

REFERENCES

- (1) GRODINS, F. S., S. L. OSBORNE AND A. C. IVY. Arch. Phys. Therapy 23: 729, 1942.
- (2) FISCHER, E. This Journal 127: 605, 1939.
- (3) GUTMANN, E. AND L. GUTTMAN. Lancet 1: 169, 1942.
- (4) SOLANDT, D. Y., D. B. DELURY AND J. HUNTER. Arch. of Neurol. and Psych. 49: 802, 1943.
- (5) HINES, H. M., J. B. THOMSON AND B. LAZERE. Arch. Phys. Therapy 24: 69, 1943.
- (6) CHOR, H., D. CLEVELAND, H. A. DAVENPORT, R. C. DOLKART AND G. BEARD. Physiother. Rev. 19: 340, 1939.
- (7) MOLANDER, C. O., F. S. STEINITZ AND R. ASHER. Arch. Phys. Therapy 22: 154, 1941.
- (8) DOUPE, J., R. BARNES AND A. S. KERR. J. Neurol. and Psych. 6: 136, 1943.
- (9) MITTLEMAN, E., F. S. GRODINS, S. L. OSBORNE AND A. C. IVY. Electronics 16: 132, 1943.
- (10) LANGLEY, J. N. AND M. HASHIMOTO. J. Physiol. 52: 15, 1918.
- (11) KNOWLTON, G. C. AND H. M. HINES. Proc. Soc. Exper. Biol. and Med. 35: 394, 1936.
- (12) OSBORNE, S. L., GRODINS, F. S., E. MITTLEMAN AND A. C. IVY. Arch. Phys. Therapy, 25: 338, 1944.
- (13) BREMER, F. J. Physiol. 76: 65, 1932.
- (14) DOUPE, J. J. Neurol. and Psych. 6: 141, 1943.
- (15) KATZ, B. Electrical excitation of nerve. Oxford, Medical Publications. London, 1939, p. 44.
- (16) SKOGLUND, C. R. Acta Physiol. Scandinavica, 4: Suppl. XII, 1942.
- (17) ROSENBLUTH, A. AND J. V. LUCCO. This Journal 120: 781, 1937.
- (18) GRODINS, F. S., S. L. OSBORNE, F. R. JOHNSON AND A. C. IVY. This Journal, in press.

THE INFLUENCE OF MUSCLE PAIN ON CORTICALLY INDUCED MOVEMENTS¹

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It is well known that voluntary movements as well as movements induced by electrical stimulation of the motor cortex are greatly modified by afferent impulses. That a deafferented leg may become useless to the animal in spite of intact motor innervation was shown by Mott and Sherrington (1895) and others. Recently Mettler and Mettler (1940) demonstrated that deafferentation modified the effects of stimulation of the motor cortex inasmuch as phasic responses could not be obtained after sectioning of the posterior roots. Uchtomsky claims that visceral afferent impulses may modify cortically induced movements. The question as to the effects of pain in general, and muscle pain in particular, on cortically induced movements does not seem to have been investigated as yet. It has been shown elsewhere by the present writers (1944) that muscle pain interferes with muscular co-ordination as demonstrated by distinct disturbances in handwriting and in the finger-nose test. It was also shown that ischemic muscular pain causes the temporary disappearance of tendon reflexes in man. As a further contribution to the understanding of the effects of pain on the central nervous system the present study was undertaken.

METHODS. Experiments were performed on 12 cats in dial-urethane anesthesia (0.45 cc./kgm. intraperitoneally). The left motor cortex was exposed and stimulated with condenser discharges of varying frequencies² at a voltage of 3 to 5 volts by means of bipolar silver electrodes at intervals of two minutes. The Horsley-Clarke apparatus secured the electrodes at the desired spot. The responses remained constant over long periods of time.

As had been shown by Lewis (1942) muscle pain may be produced either through the injection of hypertonic solution into a muscle or by means of faradic currents applied to the muscle. In addition to NaCl recommended by Lewis, KCl, CaCl₂ and MgCl₂ were used, but the latter salts were not more effective than NaCl and were therefore abandoned. Apparently the effect is largely dependent on the degree of hypertonicity since it was found that 15 per cent NaCl was more effective than 6 per cent.

As an indicator of the effectiveness of the "pain" stimulation the pupillary reaction was studied. It has been shown earlier by Ury and Gellhorn (1938) that pain stimulation leads to pupillary dilatation in the cat, which is solely due to inhibition of the tone of the third nerve. This reaction was found to be very valuable in the present experiments as a measure of the degree of excitation of pain fibers.

¹ Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

² We used an apparatus designed by C. Goodwin.

RESULTS. I. *The effect of pain on the pupil.* In the investigations of Ury and Gellhorn (1938) the effect of faradic stimulation of the skin and of the sciatic nerve caused a dilatation of the pupil which was present in the normal as well as in the sympathectomized pupil. Since these stimuli failed to alter the diameter of the parasympathectomized pupil the dilatation was interpreted as being due to an inhibition of the tone of the oculomotor nerve. Stimulation of the viscera by distention likewise caused pupillary dilatation which seemed to be due to inhibition of the parasympathetic nerve fibers. (McSwiney and collaborators, 1935-37.) In view of the close relation of muscle and visceral pain (Lewis, 1942) it was expected that muscle pain would likewise cause pupillary dilatation. This was confirmed in experiments in which either NaCl or faradization of the muscle was used. Depending on the degree of narcosis and the sensitivity of the animal, pupillary dilatation of varying degrees and duration occurred. In general, it was found that pain induced by NaCl persisted for several minutes whereas faradization of the muscle rarely lasted longer than one minute. By using various muscles these effects could be repeated many times in the same animal.

It was likewise found that faradization of the diaphragm which is accompanied by a referred pain similar to that observed on stimulation of visceral nerves, caused a dilatation of the pupil. A few observations indicated that injection of lactic acid into the femoral artery, which was known to cause distinct pain (Burget and Livingston, 1931), also caused dilatation of the pupil. Since we found that electrical stimulation of cutaneous nerve fibers (n. saphenous) was likewise accompanied by pupillary dilatation and since in all these observations the pupillary dilatation occurred to a similar degree on the normal as well as on the sympathectomized pupil it may be said that stimulation of pain fibers of the skin, muscles, viscera and arteries causes pupillary dilatation through inhibition of the parasympathetic. The degree of pupillary dilatation is apparently a reliable indicator of the effect exerted on the central nervous system. If the pupillary dilatation is great it is ordinarily accompanied by vocalization and alteration in respiration. Occasionally it may be followed by movements of the extremities.

II. *The effect of muscle pain on cortically induced movements.* In all experiments reported in this section the stimuli were applied for 15 seconds on the motor cortex thereby permitting ample time for facilitation. Consequently some movements occurred practically instantaneously whereas others, due to facilitation, appeared after various intervals of the time during the period of stimulation. Further details are recorded in the individual protocols.

The effects of muscular pain on cortically induced movements were paralleled by the action of pain on the normal and sympathectomized pupils. When marked changes in the type and degree of movements induced by cortical stimulation occurred as the result of the application of "pain" stimuli to the muscles, they were accompanied by distinct dilatation of the pupils. If, however, such stimuli caused only a brief and slight pupillary dilatation, the effect on the cortex

was either completely absent or confined to the brief period in which the pupils dilated. The results obtained may be classified into several groups:

A. *Intensification of cortically induced movements.* In numerous experiments it was observed that injection of a hypertonic NaCl solution or faradization of the muscle caused a definite increase in the intensity of the movements. Thus, the stimulation of a point of the motor cortex which caused, during the control period, a flexion of the contralateral shoulder, elbow and foot, may, under the influence of NaCl, induce the same type of movements, but of a greatly increased intensity. This increase in the excitability of the motor cortex may appear not only in the form of an intensification of movements, but also in the form of after-discharge. Such an after-discharge may occur in experiments in which during control periods no after-discharge was observed, or it may be seen in the form of an after-discharge of longer duration. The effects elicited under the influence of "pain" stimuli originating in the muscles disappear gradually and the movements become similar to those observed in the control period after an interval of about 3 to 15 minutes. The effects resulting from faradization of muscles are similar but weaker, both in intensity and duration.

If, during the control period, certain movements appear after a latent period of several seconds, indicating that facilitation processes are involved in the elicitation of these movements, the effect of "pain" stimuli appears as a shortening of this latent period (table 1), suggesting that "pain" stimuli improve conditions for cortical facilitation.

It may be emphasized that in this, as well as in the two subsequent groups of experiments, the effects of pain are not restricted to the contralateral, but appear likewise in the ipsilateral cortex. The latter case as illustrated in table 2 is of importance inasmuch as it excludes the possibility that the quantitative and qualitative changes in the effects of cortical stimulation were due to local changes in the innervated muscles. The experiments described in table 2 show clearly that the injection of NaCl into the left triceps or into the left quadriceps alters the effect of stimulation of the left motor cortex on the right foreleg, although no local changes were produced in the muscles of the right foreleg.

B. *Cortical spread as a result of muscle pain.* In most experiments in which the injection of NaCl or other salts into the muscle was accompanied by marked pupillary dilatation the movements resulting from cortical stimulation not only became stronger, but also involved muscle groups not activated under control conditions. Table 3 A shows that injection of 1 cc. 8 per cent CaCl_2 into the right hamstrings caused a pupillary dilatation from 4 to 7 mm. During and shortly following this period of dilatation the stimulation of the left motor cortex elicited, in addition to a vigorous contraction of the contralateral hindleg, a flexion of the ipsilateral hip. Ten minutes after the injection this effect disappeared completely, but was elicited again by a second injection of CaCl_2 into the right hamstring muscles³. Later, in the same animal a point was stimulated which caused contraction in the facial muscles, the orbicularis oculi, and the ear on the con-

³ This part of the experiment is omitted from table 3.

tralateral side, in addition to contractions of the muscles innervating jaw and neck. NaCl injection into the right triceps led to an unusually large pupillary dilatation accompanied by vocalization. The contractions of the muscles of

TABLE 1

Unipolar stimulation of left motor cortex (6.5 mm. lateral from the midline and 1 mm. posterior to cruciate sulcus) for 15 seconds with condenser discharges (7 volts; 43/sec.)

Movements of the right (contralateral) extremity

TIME	HIP		KNEE		PUPILLARY DIAMETER
	Movement	Latent Period	Movement	Latent period	
		<i>sec.</i>		<i>sec.</i>	<i>mm.</i>
3:24	+ Abd. + Flex.	0 5	++ Flex.	5	
3:26	+ Abd. + Flex.	3 7	+ Flex.	7	
3:29	+ Abd. + Flex.	0 7	++ Flex.	7	
3:33	+ Abd. ++ Flex.	0 7	++ Flex.	7	3.5
3:34	2½ cc. 15% NaCl injected in rt. triceps				7.0
3:35	+++ Flex.	0	+++ Flex. & after discharge	0	5.0
3:37	+ Abd. +++ Flex.	0 0	+++ Flex. & after discharge	0	4.5
3:39	+ Abd. +++ Flex.	0 0	+++ Flex.	0	4.0
3:41	+ Abd. ++ Flex.	0 5	+++ Flex.	5	3.5
3:43	+ Abd. ++ Flex.	0 4	+++ Flex.	4	3.5
3:45	+ Abd. + Flex.	0 0	++ Flex.	6	
3:47	+ Abd. ++ Flex.	0 7	++ Flex.	7	3.5

ear, face and orbicularis oculi were temporarily intensified, but more striking was the appearance of a flexion of the contralateral hip 5 seconds after the onset of the stimulation. Table 3 B illustrates the intensification of a cortically induced

movement of the right foreleg following the injection of NaCl into the left triceps. Moreover, under the influence of the "pain" stimuli a movement of the left hind-

TABLE 2

Bipolar stimulation of left motor cortex (9 mm. lateral from the midline and 1 mm. posterior to cruciate sulcus) for 15 seconds with condenser discharges (3 volts; 16/sec.)

Movements of the right (contralateral extremity)

TIME	SHOULDER		ELBOW		WRIST		PUPILLARY DIAMETER
	Movement	Latent period	Movement	Latent period	Movement	Latent period	
		sec.		sec.		sec.	mm.
2:30	+++ Flex.	8	+ Flex.	8	++ Flex.	7	
2:32	+++ Flex.	8	+ Flex.	8	++ Flex.	7	Slit
2:33 ^{30"}	2 cc. of 15% NaCl injected into left gastrocnemius m.						2
2:34	+++ Flex.	4	+++ Flex.	5	+ Flex.	5	
2:36	+++ Flex.	8	++ Flex.	7	+ Flex.	5	1
2:38	+++ Flex.	8	++ Flex.	7	+ Flex.	5	Slit
2:40 ^{30"}	3 cc. of 15% NaCl injected into left hamstring m.						
2:42	+++ Flex.	7	+++ Flex.	7	+++ Flex.	5	3
2:44	+++ Flex.	7	+++ Flex.	7	+ Flex.	6	Slit
2:46	++ Flex.	7	+ Flex.	7	+ Flex.	5	Slit
2:48	++ Flex.	7	+ Flex.	7	+ Flex.	5	Slit

TABLE 3

A

Bipolar stimulation of left motor cortex (5 mm. lateral from midline and 3 mm. posterior to cruciate sulcus) for 15 seconds with condenser discharges (5.5 volts; 90/sec.)

TIME	IPSI LATERAL HIP		CONTRALATERAL HIP		CONTRALATERAL KNEE		CONTRALATERAL ANKLE		PUPIL LARY DIAMETER
	Movement	Latent period	Movement	Latent period	Movement	Latent period	Movement	Latent period	
		sec.		sec.		sec.		sec.	mm.
10:59			++ Ext.	2	+++ Flex.	2	+++ Flex.	2	4
11:01			++ Ext.	2	+++ Flex.	2	+++ Flex.	2	4
11:03			++ Ext.	2	+++ Flex.	2	+++ Flex.	2	4
11:04 ^{30"}	1 cc. of 8% CaCl ₂ injected into right hamstrings								
11:05	+ Ext.	7	++ Ext.	0	+++ Flex.	0	+++ Flex.	0	7
11:07	++ Ext.	6	++ Ext.	0	++++ Flex.	0	+++ Flex.	0	7
11:09	++ Ext.	6	++ Ext.	0	+++ Flex.	0	+++ Flex.	0	4
11:11	++ Ext.	6	++ Ext.	0	+++ Flex.	0	+++ Flex.	0	4
	diminish- ing								
11:13	+ Ext.	6	++ Ext.	0	+++ Flex.	0	+++ Flex.	0	4
11:15			++ Ext.	0	+++ Flex.	0	+++ Flex.	0	4
11:17			++ Ext.	0	+++ Flex.	0	+++ Flex.	0	4
11:19			++ Ext.	2	++ Flex.	2	++ Flex.	2	4
11:21			++ Ext.	2	++ Flex.	2	++ Flex.	2	4

leg appears on cortical stimulation. This latter effect is not completely reversible within 15 minutes, but the appearance of the movement is progressively delayed as the effect of the "pain" stimuli on the pupils disappears.

In table 4 the effect of injection of NaCl into the temporal muscle is recorded. The results of this experiment are a combination of the features discussed in this

TABLE 3

B

Bipolar stimulation of left motor cortex (8 mm. lateral from the midline and 1 mm. posterior to cruciate sulcus) for 15 seconds with condenser discharges (3 volts; 16/sec.)

Movements are contralateral

TIME	SHOULDER		ELBOW		WRIST		IPSILAT. KNEE		PUPIL-LARY DIAMETER
	Movement	Latent period	Movement	Latent period	Movement	Latent period	Movement	Latent period	
		sec.		sec.		sec.		sec.	mm.
1:50	++ Flex.	7	+ Flex.	7	++ Flex.	5			
1:58	+ Flex.	8	+ Flex.	7	++ Flex.	4			0.5
2:00	+ Flex.	8	+ Flex.	7	++ Flex.	5			0.5
2:01 ³⁰	2 cc. 15% NaCl injected into left triceps								
2:02	+++ Flex.	5	++ Flex.	8	++ Flex.	8	+ Flex.	7	3.0
2:04	++ Flex.	6	+ Flex.	6	+ Flex.	6	+ Flex.	7	2.0
2:06	+++ Flex.	8	++ Flex.	8	++ Flex.	8	+ Flex.	10	1.0
2:08	++ Flex.	6	++ Flex.	6	++ Flex.	5	+ Flex.	11	
2:10	++ Flex.	8	++ Flex.	8	++ Flex.	5	+ Flex.	12	1.0
2:12	++ Flex.	8	++ Flex.	8	++ Flex.	3	+ Flex.	14	
2:14	++ Flex.	9	++ Flex.	9	++ Flex.	4	+ Flex.	14	
2:16	++ Flex.	9	++ Flex.	9	++ Flex.	4	+ Flex.	14	

TABLE 4

Unipolar stimulation of left motor cortex (8 mm. lateral from the midline and just posterior to cruciate sulcus) for 15 seconds with condenser discharges (5.5 volts; 16/sec.)

Movements of the right (contralateral) side

TIME	NECK		SHOULDER		ELBOW		WRIST		HIP		PUPIL-LARY DIAMETER
	Movement	Latent period	Movement	Latent period	Movement	Latent period	Movement	Latent period	Movement	Latent period	
		sec.		sec.		sec.		sec.		sec.	mm.
10:39	++	5	++ Flex.	0	++ Flex.	5	+ Flex.	6			
10:41	++	5	++ Flex.	0	++ Flex.	6	+ Flex.	7			
10:43	++	5	++ Flex.	0	++ Flex.	6	+ Flex.	7			1.5
10:44 ³⁰	2 cc. of 15% NaCl injected in left temporal muscle										
10:45	++	3	+++ Flex.	0	++ Flex.	3	+ Flex.	3	+ Flex.	10	2.5
10:47	++	3	+++ Flex.	0	++ Flex.	3	+ Flex.	3	(+) Flex.	12	2.5
10:49	++	3	+++ Flex.	0	++ Flex.	3	+ Flex.	3			1.5
10:51	++	5	+++ Flex.	0	++ Flex.	5	+ Flex.	3			1.5
10:53	++	5	+++ Flex.	0	++ Flex.	5	+ Flex.	5			1.5
10:54	3 cc. of 15% NaCl in right temporal muscle										
10:55	++	0	+++ Flex.	0	++ Flex.	0	+ Flex.	0	(+) Flex.	7	3.0
10:56											2.5
10:57	++	4	+++ Flex.	0	++ Flex.	4	+ Flex.	4			1.0
10:59	++	5	+++ Flex.	0	++ Flex.	5	+ Flex.	5			1.0

and in the preceding section. The contraction of neck muscles as well as the flexion of the contralateral shoulder and foreleg appear earlier (and in the second NaCl experiment of table 4 even immediately) indicating the improvement of

facilitation under the influence of the "pain" stimuli. In addition, a flexion of the hip was also elicited although this reaction was absent under control conditions.

C. *Modification of cortically induced movements.* The most striking result of muscle pain on cortically induced movements was the observation that under the influence of these afferent impulses cortical stimulation resulted in movements which were *qualitatively* different from those seen in the preceding and the following control periods. In the first experiment of table 5 it is shown that under the influence of injection of NaCl into the left triceps cortical stimulation of the left motor area results in an extension of the right shoulder whereas under control conditions a flexion and abduction of the shoulder occurred. It is interesting to note that in the first record obtained after muscle pain had been elicited, the extension of the shoulder had completely replaced the flexion and abduction which had been seen previously. Two and four minutes later the extension was followed by a flexion which gradually increased in intensity. After two more minutes flexion and abduction occurred in their original strength, and the extension disappeared completely. During the second part of this experiment the injection of NaCl was repeated, but this time the solution was injected into the left hamstring muscles. Here again the cortically induced movement of the right shoulder was altered as in the preceding experiment, but in addition, an ipsilateral extension of the foreleg occurred under the influence of the "pain" stimuli.

The experiment recorded in table 5 is typical of a number of similar experiments inasmuch as it shows that the various effects of "pain" stimulation on the cortical response to electrical stimuli may occur simultaneously in the same experiment. Thus table 5 illustrates that in addition to the qualitative changes (substitution of the extension of the shoulder for its flexion) quantitative changes in the flexion of hip and knee may be induced at the same time.

DISCUSSION. Summarizing the results of this and a preceding paper it may be stated that "pain" stimuli originating in muscle may modify quantitatively and qualitatively the performance of movements. This statement applies to reflexly and voluntarily controlled movements as well as to movements elicited by electrical stimulation of the motor cortex. The effects are reversible and are attributed to the modifying influence of increased afferent discharges on the excitability of various parts of the central nervous system. The changes in the somatic nervous system are accompanied by autonomic changes as indicated by the pupillary dilatation which is due to an inhibition of the tone of the third nerve. Intensity and duration of the somatic effects are paralleled by these autonomic changes.

In the experiments reported in an earlier paper it was shown that pain elicited in the muscle of hand and fingers through ischemic contractions altered muscular co-ordination as shown by disturbances in handwriting. However, the handwriting with the other hand was not altered. Likewise, it was found that tendon reflexes were abolished by pain originating in the effector muscle. The experiments described in this paper show that the action of muscle pain on the central nervous system may be more widespread than is suggested by our earlier obser-

TABLE 5

Bipolar stimulation of left motor cortex (8 mm. lateral from midline, and 1 mm. posterior to cruciate sulcus) for 15 seconds with condenser discharges (5.5 volts: 16/sec.)

Movements are contralateral unless indicated

TIME	NECK		SHOULDER		ELBOW		HIP		KNEE		PUPIL- LARY DIAMETER
	Movement	Latent period sec.	Movement	Latent period sec.	Movement	Latent period sec.	Movement	Latent period sec.	Movement	Latent period sec.	mm.
11:11	++	0	+++ Flex. + Abd.	0 0	++ Flex.	0					2
11:13	++	0	+++ Flex. + Abd.	0 0	++ Flex.	0					2
11:15	++	0	+++ Flex. + Abd.	0 0	++ Flex.	0					2
11:17 ^{40"}	3 cc. 15% NaCl injected into left triceps m.										
11:18	++	0	+ Ext.	0	++ Flex.	0	+ Flex.	7			7
11:20	++	0	+ Ext. +++ Flex.	0 3.	++ Flex.	3	+ Flex.	7	+ Flex.	7	7
11:22	++	0	+ Ext. +++ Flex.	0 5	++ Flex.	5	+ Flex.	7	+ Flex.	7	
11:24	++	0	+++ Flex. + Abd.	0	++ Flex.	0	(+) Flex.	7	(+) Flex.	7	4
11:26	++	0	+++ Flex. + Abd.	0 0	++ Flex.	0	(+) Flex.	10	(+) Flex.	10	
11:28	++	0	+++ Flex. + Abd.	0 0	++ Flex.	0	(+) Flex.	13	(+) Flex.	13	
11:30	++	0	+++ Flex. + Abd.	0	++ Flex.	0	(+) Flex.	11			3.5
11:31 ^{45"}	3 cc. 15% NaCl injected into left hamstring m.										
11:32	++	0	+++ Flex. ++ Ext. & ip- silateral + Ext.	3 0 5	++ Flex.	0	+ Flex.	9	+ Flex.	9	8
11:34	++	0	++ Ext. +++ Flex. ipsilateral ++ Ext.	0 5 5	+++ Flex. ipsilateral + Ext.	3 5					
11:36	++	0	+ Ext. +++ Flex. Ipsi. + Ext.	0 4 4	+++ Flex.	4	(+) Flex.	10	(+) Flex.	10	
11:38	++	0	+++ Flex. + Abd.	0 0	++ Flex.	3					
11:40	++	0	+++ Flex. + Abd.	0 0	++ Flex.	3					4.5
11:43	+	0	++ Flex. + Abd.	0	++ Flex.	0					4.5
11:45	+	0	++ Flex. + Abd.	0	++ Flex.	0					4.5

uations on man. The fact that NaCl injections into muscles of the left hindleg may qualitatively and quantitatively alter the effects resulting from stimulation of the left motor area indicates that these afferent impulses influence cortical excitability in both hemispheres. It is assumed that this cortical spread is due to the greater intensity of the stimuli used.

Gasser (1937) states that the direction which afferent impulses follow is variable and depends on "the situation obtaining at the moment." A similar statement is justified with respect to efferent impulses. The basis for this statement is not only the experiments described in this paper but also the investigations of Sherrington (1895), Mettler (1940), Uchtomsky (1925) and Magnus (1910), who showed that the reflex action as well as the effect of cortical stimulation is determined by afferent impulses.

SUMMARY

Experiments are reported in which the effect of afferent "pain" stimuli on the cortex of anesthetized cats is investigated. Procedures such as injection of hypertonic NaCl into a muscle or of faradization of a muscle, which cause muscle pain in man, are used as stimuli. These stimuli cause pupillary dilatation due to an inhibition of the tone of the third nerve and in some instances cause vocalization. Under their influence the effect of stimulation of the motor cortex is either intensified or qualitatively altered so that different movements may be substituted temporarily for those obtained under control conditions. The action of these afferent impulses is not limited to the contralateral cortex, but may likewise modify the reactivity of the ipsilateral cortex to electrical stimulation.

REFERENCES

- BURGET, G. E. AND W. K. LIVINGSTON. *This Journal* 97: 249, 1931.
GASSER, H. S. *Bull. N. Y. Acad. of Med.* 13: 324, 1937.
GELLHORN, E. AND L. THOMPSON. *In press.*
HARPER, A. A., B. A. McSWINEY AND S. F. SUFFOLK. *J. Physiol.* 85: 267, 1935.
IRVING, J. T., B. A. McSWINEY AND S. F. SUFFOLK. *J. Physiol.* 89: 407, 1937.
LEWIS, T. *Pain*. New York, 1942.
METTLER, F. A. AND C. METTLER. *J. Neurophysiol.* 3: 527, 1940.
MOTT, F. W. AND C. S. SHERRINGTON. *Proc. Roy. Soc.* 57: 481, 1895.
UCHTOMSKY cf. J. M. UFLAND. *Pflüger's Arch.* 208: 87, 1925.
URY, B. AND E. GELLHORN. *Proc. Soc. Exper. Biol. and Med.* 38: 426, 1938.
MAGNUS, R. *Pflüger's Arch.* 130: 253, 1909; 134: 554, 584, 1910.

FASTING AND GLUCONEOGENESIS IN THE KIDNEY OF THE EVISCERATED RAT¹

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Reinecke (1) demonstrated that glucose is added to the blood as it flows through the kidney of the eviscerated rat. It was further noted by Reinecke and Roberts (2) that the eviscerated rat which had previously been fasted two to four days maintained its blood sugar much better than animals fed right up to the time of operation; nephrectomy apparently eliminated this difference.

The present study indicates that the ability of the fasted animal to maintain its blood sugar level after evisceration is, in part, due to an acceleration of gluconeogenesis in the kidney. This acceleration apparently begins soon after the deprivation of food and continues for at least two weeks thereafter.

METHODS. The animals used in all the experiments reported below were Sprague-Dawley male albino rats, weighing between 300 and 350 grams in the fed state. Purina fox chow was fed *ad libitum*.

Those animals which were later to be eviscerated were treated and maintained as previously described (1, 2). "Fed" animals were kept in cages with food and water up to the time of evisceration, or, in the case of the intact animals, until blood samples were taken for arterio-venous differences. The methods of evisceration and of obtaining simultaneous aortal and renal vein blood samples were essentially those of Reinecke (1). All blood analyses, except for hemoglobin, were carried out on dilute tungstic acid filtrates of whole blood. Total blood "sugar" was estimated by the method of Reinecke (3); this includes reducing substances other than glucose. Fermentable reducing substance was determined by the methods of Reinecke (1) and of Roberts, *et al.* (4) and presumably represents mainly true glucose. The procedure of Frame *et al.* (5) was used in estimating blood amino acid nitrogen. The Evelyn photo-electric colorimeter was employed in the determination of oxyhemoglobin in aortal and renal vein blood samples (6).

The blood sugar curves of the eviscerates were constructed from analyses of 0.02 cc. samples of blood from the tail vein. All arterio-venous (A-V) samples were obtained from animals injected subcutaneously 15 minutes earlier with an anesthetic dose of sodium amytal (10 mgm. per 100 gram body weight). In the case of the eviscerates, A-V samples were obtained 6 hours after operation.

Oxygen consumption rates of the eviscerated animals were measured about 5 hours after operation by means of the simple manometric device previously outlined (4).

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RESULTS. It was found that the kidneys of fasted eviscerated rats added considerable amounts of glucose to the blood, whereas those of fed animals,

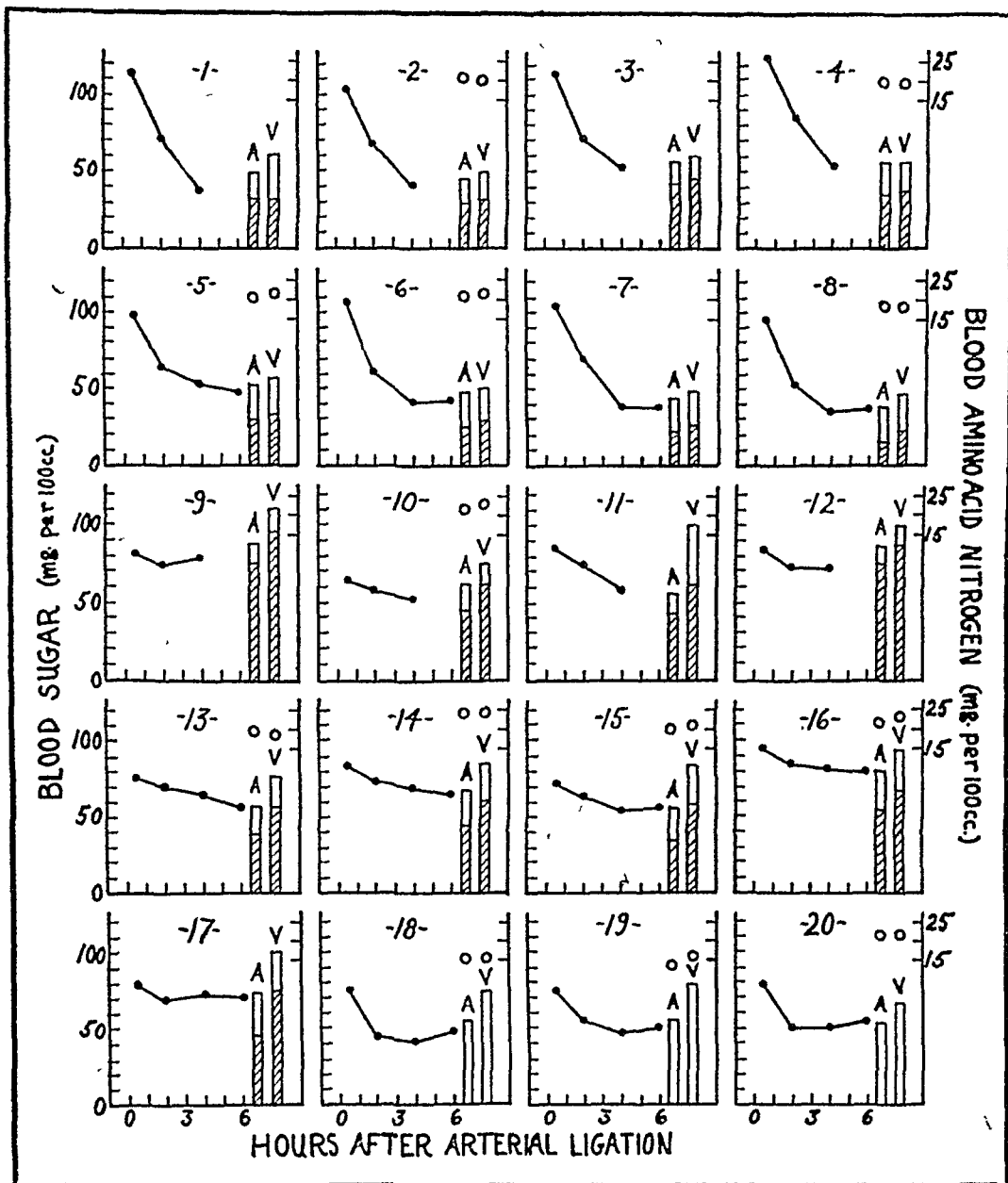


Fig. 1. The effect of fasting on the output of glucose by the kidney of the eviscerated rat. Animals 1 to 8 were fed up to the time of evisceration; 9 to 17 were fasted 2 days; 18 to 20 were fasted 2 weeks. The blood sugar curves after evisceration were constructed from values obtained by analysis of periodic tail blood samples for total "sugar". The heights of the bars represent the total "sugar" (entire bar) and the fermentable sugar (cross-hatched portion) found in the lower aorta, A, and in the left renal vein V, 6 hours after tying off the coeliac and mesenteric arteries. The open circles above the bars indicate the amount of amino acid nitrogen found in similar arterial and venous samples taken at the same time.

similarly treated, added little or none. Thus, as figure 1 and table 1 reveal, the amount of sugar added to the blood passing through the kidneys of 12 rats pre-

viously fasted 2 days, and of 3 rats fasted 2 weeks, was between 15 and 25 mgm. per cent; the corresponding value for 12 fed animals was about 5 mgm. per cent. Wherever measured, the differences in fermentable reducing substance, presumably true glucose, were quite similar to the differences in total "sugar". Hemo-

TABLE 1
Renal arterio-venous differences in eviscerated rats†*

	AORTA 1	RENAL VEIN 2	DIFFERENCE 2 - 1	S.D. OF DIFFERENCE	NO. OF RATS
Fed animals					
Total "sugar" (mgm./100 cc.).....	46 (39 to 55)	51 (45 to 58)	5 (0 to 8)	±2.4	12
Ferm. sugar (mgm./100 cc.) ..	28 (16 to 41)	31 (22 to 44)	3 (-1 to 6)	±2.1	8
Amino N (mgm./100 cc.)....	21 (19 to 22)	20 (18 to 22)	-1 (-1 to 0)		6
Hemoglobin (gram/100 cc.)...	17	17	0		3
Fasted 2 days					
Total "sugar" (mgm./100 cc.).....	65 (54 to 88)	86 (75 to 110)	21 (13 to 27)	±5.1	12
Ferm. sugar (mgm./100 cc.) ..	48 (39 to 74)	66 (57 to 94)	18 (13 to 30)	±5.1	8
Amino N (mgm./100 cc.)....	23 (19 to 24)	24 (18 to 24)	1 (-1 to 2)		6
Hemoglobin (gram/100 cc.)...	17 (16 to 17)	17 (16 to 17)	0 (-1 to 1)		3
Fasted 2 weeks					
Total "sugar" (mgm./100 cc.).....	55 (53 to 56)	72 (66 to 77)	17 (13 to 19)		3
Amino N (mgm./100 cc.)....	18 (15 to 22)	18 (16 to 22)	0 (0 to 1)		3

* Average values and ranges (in parentheses) are shown.

† Simultaneous blood samples were obtained from the lower aorta and left renal vein of amylal anesthetized rats, 6 hours after evisceration.

globin values on a number of samples demonstrated the lack of a measurable hemo-concentration by the kidney, so that this could not be a factor.

Figure 2 indicates that there was no significant difference in the oxygen consumption rates, taken 5 hours after evisceration, of 6 fed animals and 8 animals fasted 2 days. The difference in sugar production in these two groups, then, was not associated with any large difference in oxidative metabolism. It is interesting to note, moreover, that although sugar production was observed in

animals fasted 2 weeks prior to evisceration, total oxidative metabolism was significantly depressed. The oxygen consumption rate has been expressed both as the total rate of oxygen consumption and as the rate of oxygen consumption per unit of body surface. The latter figures are open to error because of the wide differences in the body contour of the different groups of rats. Similar results, however, were obtained by both methods of calculation.

In an attempt to discover the source of the added glucose, a number of A-V samples were analyzed for amino acid nitrogen. Figure 1 (open circles) and table

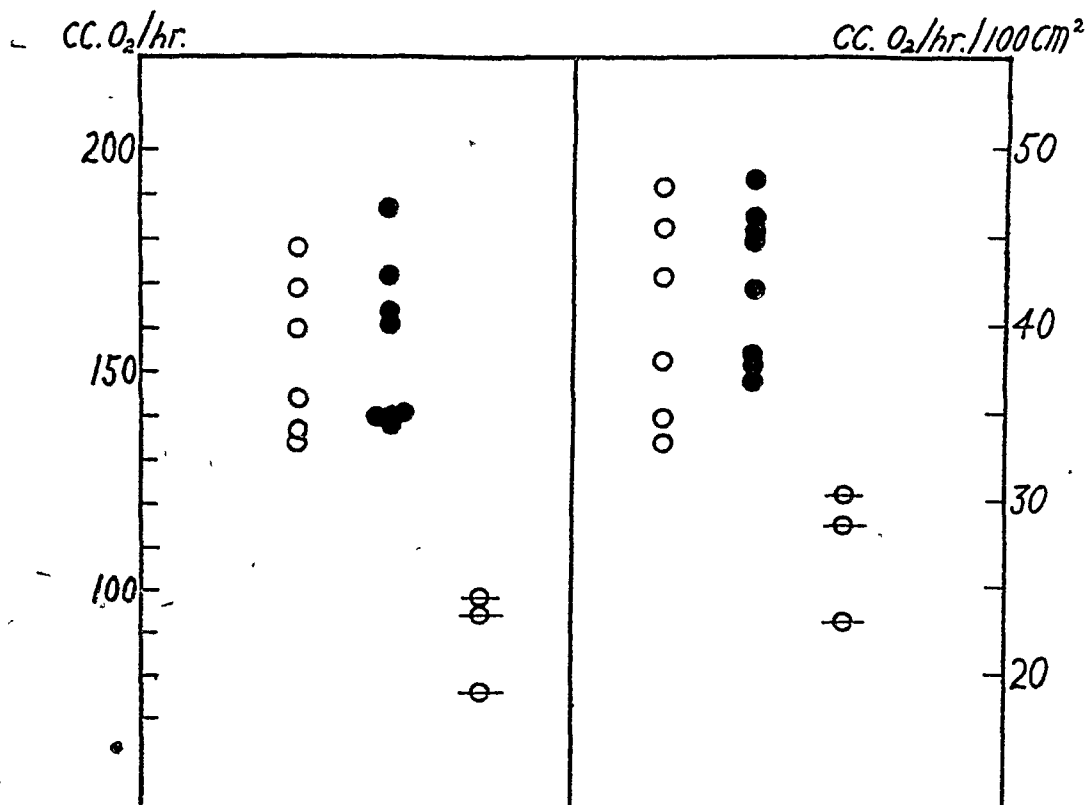


Fig. 2. The effect of fasting on the energy metabolism of the eviscerated rat.

All determinations were made 5 hours after arterial ligation incident to evisceration. The metabolism of each animal studied is expressed in two ways: total oxygen consumption rate, and oxygen consumption rate per unit body surface. Fed animals are represented by open circles, animals fasted 2 days before evisceration by closed circles, and animals fasted 2 weeks by open circles with horizontal bars.

1 seem to indicate that blood amino acids do not enter into a gluconeogenic process in the kidney, at least under the conditions studied.

The addition of glucose to the blood by the kidneys of intact rats subjected to similar periods of fasting could not be demonstrated by the A-V technique. Table 2 gives the blood sugar and blood amino acid nitrogen concentrations in the aorta and renal vein of 5 fed animals, 3 animals fasted 3 days, and of 5 animals fasted 16 days. One animal whose blood sugar had been markedly depressed by treatment with 1 unit of protamine zinc insulin daily for 3 days likewise showed no A-V difference in either blood sugar or blood amino acid nitrogen.

DISCUSSION. From the above results it appears that the ability of the fasted eviscerated rat to maintain its blood sugar is, to an appreciable extent, dependent upon a stimulation of gluconeogenesis in the kidney. Since Reinecke (1), studying eviscerated rats which were fasted for 24 hours before operation, found renal A-V glucose differences somewhat lower than those obtained above in 2 and 14 day fasted animals, but still quite large, it appears that this stimulation

TABLE 2
Simultaneous renal arterio-venous values in intact rats†*

	AORTA 1	RENAL VEIN 2	DIFFERENCE 2 - 1	NO. OF RATS
Fed animals				
Total "sugar" (mgm./100 cc.).....	99 (95 to 110)	98 (92 to 110)	-1 (-3 to 5)	5
Amino N (mgm./100 cc.)....	10 (10 to 11)	10 (9 to 11)	0 (-1 to 1)	5
Fasted 3 days				
Total "sugar" (mgm./100 cc.).....	94 (82 to 105)	97 (84 to 110)	3 (2 to 5)	3
Amino N (mgm./100 cc.)..	10 (9 to 11)	9 (9 to 10)	-1 (-1 to 0)	3
Fasted 16 days				
Total "sugar" (mgm./100 cc.).....	73 (57 to 79)	73 (57 to 80)	0 (-4 to 3)	5
Amino N (mgm./100 cc.)..	10 (8 to 14)	10 (8 to 15)	0 (-1 to 1)	5
Given prot. zn. insulin				
Total "sugar" (mgm./100 cc.).....	30	31	1	1
Amino N (mgm./100 cc.)....	6	6	0	1

* Average values and ranges (in parentheses) are shown.

† Simultaneous blood samples were obtained from the lower aorta and left renal vein of amytal anesthetized rats.

occurs quite early in the fasting process. These results suggest that the conditions which bring about an increased gluconeogenesis in the liver of the intact animal early in fasting are operative in the eviscerated animal, and are able to stimulate gluconeogenesis in the kidney of this preparation.

The amino acid nitrogen determinations reported above are not in line with the observations of Russell and Wilhelmi (7) that extra carbohydrate can be formed

"in vitro" by kidney tissue from added amino acids. Apparently, blood amino acids are not so utilized "in vivo", at least under the conditions studied.

Until further study has been made of the precursor of kidney-formed glucose and of the control of its formation, it is impossible to ascertain how fasting results in a stimulation of kidney glucogenesis. Increased availability of the precursor, or increased production of some endocrine factor may be suggested. In the latter connection, it may be noted that Russell and Wilhelmi (7) observed that the "in vitro" conversion of amino acids to glycogen was depressed in kidney slices obtained from adrenalectomized rats.

SUMMARY

Fasting resulted in an addition of glucose to the blood by the kidney of the eviscerated rat, as determined by analyses of simultaneous aortal-renal vein samples for glucose. This phenomenon appears to be at least partly responsible for the ability of the fasted rat to maintain high levels of blood sugar for many hours after evisceration.

There was no appreciable difference in the total oxidative metabolism of eviscerated animals, either fed or fasted 2 days; but animals fasted 2 weeks before evisceration showed a definite depression in oxygen consumption rate, although sugar production by the kidney was still occurring.

The precursors of the glucose added to the blood flowing through the kidney do not seem to be blood amino acids.

Glucogenesis could not be demonstrated in the kidney of intact animals subjected to similar periods of fasting.

REFERENCES

- (1) REINECKE, R. M. This Journal **140**: 276, 1943.
- (2) REINECKE, R. M. AND S. ROBERTS. This Journal **141**: 476, 1944.
- (3) REINECKE, R. M. J. Biol. Chem. **143**: 351, 1942.
- (4) ROBERTS, S., L. T. SAMUELS AND R. M. REINECKE. This Journal **140**: 639, 1944.
- (5) FRAME, E., J. A. RUSSELL AND A. E. WILHELMI. J. Biol. Chem. **149**: 255, 1943.
- (6) EVELYN, K. A. J. Biol. Chem. **115**: 63, 1936.
- (7) RUSSELL, J. A. AND A. E. WILHELMI. J. Biol. Chem. **140**: 747, 1941.

THE DIURETIC EFFECT OF GELATIN SOLUTIONS¹

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In a study of the intravenous administration of gelatin (1) in normal dogs it was noted that during a 6 hour period following the gelatin injection there was an increased urine flow. It was thought that this effect might represent a diuretic action of gelatin, but with the data available it was not possible to distinguish between the effect of gelatin and that due to the volume of fluid injected.

Knowlton (2) reported that when saline and 5 per cent saline-gelatin solutions were given alternately to rabbits, the diuretic effect of saline was inhibited by the saline-gelatin. Hogan (3) also reported that 1.5 to 2.5 per cent gelatin solutions do not yield an increased urine flow in rabbits.

The experiments reported here were designed to compare the diuretic effect of: *a*, gelatin injection with the injection of an equal volume of 0.9 per cent sodium chloride; *b*, unautoclaved gelatin with autoclaved gelatin; *c*, gelatin with a known diuretic, salyrgan-theophylline; *d*, gelatin plus salyrgan-theophylline with each alone.

PROCEDURE. Food and water were withdrawn from the dogs the evening before the day of the experiment. The animals were unanesthetized and were trained to lie quietly with a minimum of restraint. They were catheterized, the bladder was emptied, and control urine collections were made at 15 minute intervals for 1 to 2 hours. The fluid was rapidly injected intravenously by syringe and was equivalent in volume to 1 per cent of the body weight. The gelatin² solutions contained 6 grams of gelatin in 100 cc. of 0.9 per cent sodium chloride. Two preparations of autoclaved gelatin were used, one prepared by the manufacturer (lot W3-20) and one prepared in this laboratory from stock gelatin (lot B78-1); in both cases the duration of autoclaving was 20 minutes. In some experiments the dogs were given an intramuscular injection of 0.06 cc. per kgm. of a solution of salyrgan-theophylline, each cubic centimeter containing 100 mgm. salyrgan and 50 mgm. theophylline. This injection was preceded immediately either by an injection of 0.9 per cent sodium chloride solution or unautoclaved gelatin.

Urine was collected at 30 minute intervals throughout the experimental period, which was terminated when the rate of urine flow had returned to the level of the control rate and had remained at this level usually for one hour. With

¹ This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

² The gelatin was furnished by Knox Gelatine Company.

unautoclaved gelatin the duration was usually 9 hours and with other substances 6 hours.

The experiments extended through the summer and fall months, so that there was considerable variation in room temperature and humidity. To prevent the introduction of a systematic error due to these variables, the experiments were done in rotation. An attempt was made to maintain room temperature approximately constant, and in only a few instances did the animals pant.

In a few experiments all of the urine was collected for 6 hours following the injection of autoclaved and unautoclaved gelatin. The total gelatin excretion during this period was determined using a previously reported method (1).

Occasionally experiments were discarded because either the animal was quite restless, was nauseated following the injection of saline or gelatin, or the urine was bloody.

RESULTS. In table 1 the results are expressed as the diuretic ratio, $\frac{\text{net urine output in cc.} \times 100}{\text{volume of fluid injected in cc.}}$. The net urine output was calculated by subtracting from the total urine output during the experimental period the quantity which would have been excreted during this period had nothing been injected. This latter quantity is based on the assumption that the control rate of urine flow may be extrapolated through the experimental period. The value of expressing the results in this manner rather than as the rate of urine excretion is that the diuretic ratio is a measure of the effect of these substances on the fluid balance of the animal.

It will be seen that with saline and autoclaved gelatin approximately 50 per cent of the fluid injected was recovered as urine during the period of elevated rate of urine excretion. With unautoclaved gelatin, salyrgan-theophylline and a combination of these two more fluid was recovered as urine than was injected.

In figure 1 a typical response to these substances is given. It will be noted that the peak of excretion is reached somewhat later with gelatin than with saline or salyrgan-theophylline, and that with unautoclaved gelatin the duration of increased excretion is longer. During the first half-hour following unautoclaved gelatin the urine excretion fell below the control level, which was a frequent finding with this substance.

Five experiments each were run with autoclaved and unautoclaved gelatin to determine the excretion of gelatin in urine during a 6 hour period following injection. With autoclaved gelatin from 10 to 21.4 per cent (average 17.7 per cent) of the gelatin injected was excreted, while with unautoclaved gelatin from 9.5 to 14 per cent (average 12.2 per cent) was excreted. This difference is not statistically significant.

DISCUSSION. The correlation between body weight and control urine flow in 105 determinations on 10 dogs was found to be $r = 0.559 \pm 0.082$ (standard error). This is interpreted as indicating that extraneous factors such as fluid balance, temperature, etc., were well controlled within natural limitations.

For gelatin to be considered an effective diuretic it is necessary that the volume of urine recovered, when corrected for the extrapolated control urine flow, shall

TABLE 1

Per cent of volume of fluid injected recovered as urine, corrected for extrapolated control urine

$$\text{flow, } \left(\frac{\text{net urine output in cc.} \times 100}{\text{fluid injected in cc.}} \right)$$

0 = 0.9 per cent sodium chloride, A = autoclaved gelatin, G = unautoclaved gelatin,
S = salyrgan-theophylline, SG = salyrgan-theophylline-unautoclaved gelatin

DOG	O	A	G	S	SG
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	0 13 11	44 42 69	270 336		
2	13 -9 39	215 47 0 -44	260 161		
3	0	50 18 31	245	156 185	90 161
4	41 3 80	7 20 58 70 -12	199 106 173 74		
5	85 83 36	75 55 34 57	107 173	321 415	267 247
6	117 19 94	99 117 55	221 180 241 88 154 13	268 394 210	226
7	98 162 67	43 55 86	128 185	159	
8	9 53 120	28 35 42	224	300 238	
9	11 60 19	66 17 184	172 113 130	165 292	
10	49 50	43	146 125	198 198	
Mean±.....	49.0	53.3	169.0	219.9	198.2
No. n.....	27	32	25	14	5
Standard error ±.	8.46	8.78	14.21	22.64	32.39

significantly exceed the volume of fluid injected; or in other words the diuretic ratio shall be significantly greater than 100. Saline injections were used to determine the effect of the dispersion fluid alone on urine recovery. In table 1 are listed the means for the 5 experimental groups and their estimated standard errors. Whether the net urine output following the injection of each substance is significantly greater or less than the volume of fluid injected may be judged by comparing the absolute value of $100-\bar{x}$ with the standard error $s_{\bar{x}}$. For example, the mean diuretic ratio for unautoclaved gelatin exceeds 100 per cent by 69 per cent; this difference is 4.9 times the standard error and is far greater than one which could be explained by sampling variation. The deviations from 100 per cent are likewise significant at the 0.01 probability level for all other

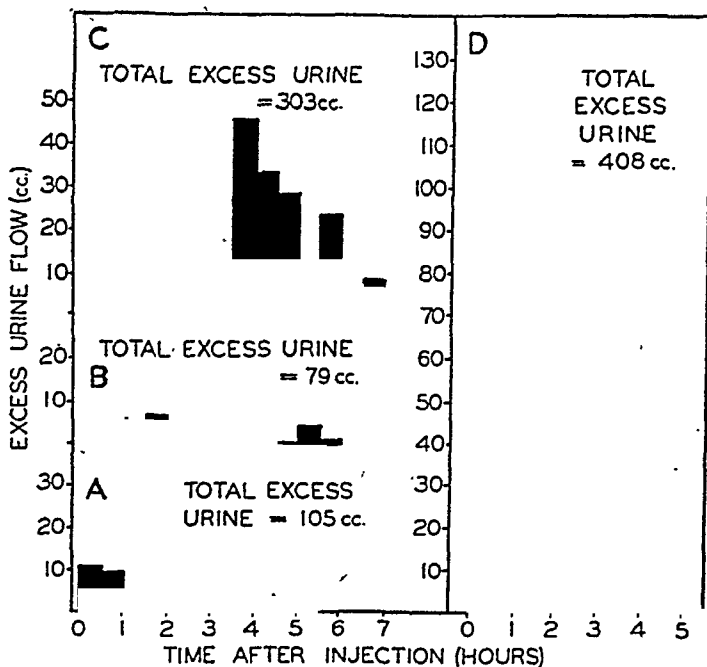


Fig. 1. Rate of urine flow in excess of the extrapolated control rate following the injection of: A = 0.9 per cent sodium chloride, B = autoclaved gelatin in saline, C = unautoclaved gelatin in saline, D = saline plus salyrgan-theophylline. Dog 10.

groups, excepting salyrgan-theophylline-unautoclaved gelatin, which, being based on only 5 tests is significant only at the 0.05 probability level. A diuretic effect as herein defined therefore has been conclusively demonstrated for unautoclaved gelatin, for salyrgan-theophylline, and for a combination of the two. The injection of saline or autoclaved gelatin, on the other hand, was accompanied by a significant degree of retention of the fluid. Undoubtedly the urine output with the last two substances remained slightly elevated above the control level for a prolonged time thus restoring the fluid balance, but this elevation was not detectable by the method of measurement we used.

Another question of interest is the significance of the differences within the group of experiments in which diuresis was produced and within the non-diuresis group. For this purpose the data of table 1 have been examined statistically

by the method of *analysis of variance* (4), and the results are presented in table 2. The variance (squared standard deviation) for the entire series of 103 diuretic ratios has been analyzed into two components, one measuring the variation among the means of the 5 experimental groups, and the other measuring the variation within the groups. The latter is further divided into variance between and within sets of measurements on individual dogs. This last component, representing the residual variation within tests involving the same dog and experimental treatment, constitutes the "experimental error." The last column of the table shows the ratio of the variance between groups to the error variance to be $F = 52.24$, while that for animal differences amounts to $F = 1.78$. There is therefore evidence that the 5 substances produce significantly different effects upon urine output. Also, as might be expected, there is evidence of individual animal differences in addition to those associated with body weight, which were controlled experimentally by the injection of varying volumes of fluid.

TABLE 2
Analysis of variance of diuretic ratio

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	VARIANCE	F
Between groups.....	4	601565	150391	52.24†
Within groups				
Between dogs.....	35	180842	5167	1.79*
Within dogs (error)....	63	181366	2879	
Total.....	102	963773		

* Probability of chance occurrence <0.05 .

† Probability of chance occurrence <0.01 .

Table 2 further provides the basis for testing the significance of the differences between the means of the 5 experimental groups. For this purpose, the relative deviate

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{V\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$

expressing the ratio of the mean difference, $\bar{x}_1 - \bar{x}_2$ to its standard error, may be computed in each case, using the error variance of table 2 ($V = 2879$) in conjunction with the sizes of the samples, n_1 and n_2 . The differences between the means for all 10 group comparisons, together with their t values, and probabilities of chance occurrence, are summarized in table 3.

All differences between means are significant with the exception of these comparisons: salyrgan-theophylline with salyrgan-theophylline-unautoclaved gelatin, salyrgan-theophylline-unautoclaved gelatin with unautoclaved gelatin, and autoclaved gelatin with saline. This means that in normal unanesthetized dogs the diuretic effect of unautoclaved gelatin is significantly less than that of

salyrgan-theophylline, while a combination of the two is not significantly greater than either alone. The effect of autoclaving is to remove the diuretic effect of gelatin, so that its effect is not significantly greater than that of saline alone.

The fact that unautoclaved gelatin and salyrgan-theophylline were also administered in combination permits the analysis of a possible interaction of these substances in their effect upon urine flow. Since the diuretic effect of each of the substances tested has a component due to the injection of saline (O), the net effects of unautoclaved gelatin (G), salyrgan-theophylline (S) and the combination of the last two (SG) are measured by $(\bar{G} - \bar{O})$, $(\bar{S} - \bar{O})$, and $(\bar{SG} - \bar{O})$, respectively, where \bar{O} , \bar{S} , \bar{G} and \bar{SG} represent the means for the four experimental groups. If, now, the effects of (G) and (S) are independent, the effect of their combined injection ($\bar{SG} - \bar{O}$) is expected to equal the sum of their separate injections $(\bar{G} - \bar{O}) + (\bar{S} - \bar{O})$, or, simplifying, $(\bar{SG} + \bar{O})$ is expected to equal

TABLE 3

Comparison of the difference between group means. Key same as in table 1

COMPARISON	MEAN DIFFERENCE	<i>t</i>	PROBABILITY
	<i>per cent</i>		
S-SG	51.7	1.85	>0.05
S-G	81.0	4.52	<0.01
S-A	196.6	11.44	<0.01
S-O	200.9	11.37	<0.01
SG-G	29.2	1.11	>0.05
SG-A	144.9	5.62	<0.01
SG-O	149.2	5.71	<0.01
G-A	115.7	8.08	<0.01
G-O	120.0	8.06	<0.01
A-O	4.3	0.31	>0.05

$(\bar{S} + \bar{G})$. An interaction or non-additive effect of the two substances in combination will therefore be measured by the difference

$$(\bar{SG} + \bar{O}) - (\bar{S} + \bar{G}) = -171.7 \text{ per cent}$$

That this difference is statistically significant may be demonstrated by comparing its appropriate variance, 85637, after adjustment for non-proportionality in the numbers of animals tested in the four groups (4), with the error variance of table 2. The variance-ratio, $F = 29.7$, is much larger than the value exceeded by chance in 1 per cent of trials. This discrepancy may represent an antagonism between the gelatin and salyrgan-theophylline, or the same mechanism of action for both, when the upper limit of a dosage-effect curve has been approached by the concentration of the two substances injected separately.

In the absence of knowledge about the mechanism of the diuretic action of gelatin one can speculate only as to the difference in the effect brought about by autoclaving. It is known that autoclaving causes some hydrolysis of the protein, and it seems likely that the lack of diuretic action after autoclaving is associated with a decrease in the average molecular weight. Since these animals

presumably had a normal plasma colloid osmotic pressure, it is possible that in the presence of a diminished plasma protein concentration gelatin which had been autoclaved might be effective.

The difference between our results and those of Knowlton may be explained by the initial decrease in urine flow following gelatin injection. Knowlton alternated the injections of saline and saline-gelatin at intervals of a few minutes, and therefore would have seen only the immediate effect of gelatin which is usually antidiuretic and not the delayed effect.

SUMMARY

It has been shown in normal unanesthetized dogs that the intravenous injection of solutions of 0.9 per cent sodium chloride or autoclaved 6 per cent gelatin in saline produces an increase in urine flow which returns to the control level usually within 6 hours. Approximately 50 per cent of the fluid injected is recovered as excess urine during this period.

Unautoclaved gelatin or salyrgan-theophylline-saline gave a marked increase in urine flow lasting about 9 and 6 hours respectively. With the former the mean value for the excess urine flow is 169 per cent of the volume injected, while with the latter it is 250 per cent. In 5 experiments a combination of these two gave a value which was significantly less than that which would have been expected if the two effects were independent.

It is concluded that 6 per cent unautoclaved gelatin is a diuretic substance when given intravenously.

REFERENCES

- (1) LITTLE, J. M. AND J. T. DAMERON. *This Journal* **139**: 438, 1943.
- (2) KNOWLTON, F. P. *J. Physiol.* **43**: 219, 1911.
- (3) HOGAN, J. J. *J. A. M. A.* **64**: 721, 1915.
- (4) SNEDECOR, G. W. *Statistical methods*. The Iowa State College Press, Ames, Iowa, 1940.

WORK IN THE HEAT AS AFFECTED BY INTAKE OF WATER, SALT AND GLUCOSE¹

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The performance of physical work in the heat can be affected for better or worse by a variety of factors, the most important being physical fitness and acclimatization, nutritional state, clothing and drugs. This paper deals with the effects of water, salt and glucose, with principal emphasis upon the attainment of the best possible performance. The effects of water soluble vitamins will be mentioned briefly. The present material will be restricted in two ways. First, attention will be confined to performance of fixed tasks by fully acclimatized men working intermittently in moist or in dry heat. Therefore, the results are applicable to steel workers, miners and soldiers in the desert, all of whom usually do a day's work in the heat and spend the rest of the time in cooler surroundings. Second, we shall consider only the immediate hour to hour effects of the above dietary factors upon men whose overall daily intake was always adequate in water, salt, carbohydrate and vitamins. There will be no consideration of prolonged deficiency or excess from day to day.

METHODS. Experiments were performed in the late fall or winter in a heated room under hot dry (100 F., 30 per cent relative humidity) and hot moist conditions (95 F. and 90 F., 83 per cent relative humidity). Six healthy young men, fully acclimatized as judged by the criteria of Robinson and colleagues (1943), marched at least three times a week at 3.5 m.p.h. up grades which will be specified in the tables. Depending upon the severity of the temperature and humidity, which were constant in individual series of experiments, they marched anywhere from one to six hours with a ten minute rest in each hour. Certain measurements were made periodically in every experiment. These were: *a*, environmental temperature and humidity with a sling psychrometer; *b*, pulse rate by palpation; *c*, rectal temperature with a calibrated clinical thermometer; *d*, sweating by net change in nude body weight. Other measurements were made in some, but not all, experiments. These were: *e*, respiratory exchange by collection of expired air in a Tissot gasometer after the subject was in a steady state, analysis for carbon dioxide and oxygen with the Haldane apparatus, and calculation of the oxygen consumption by standard procedures (Haldane, 1934; Peters and Van Slyke, 1932); *f*, protein by the micro Kjeldahl method of Ma

¹ The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the President and Fellows of Harvard College.

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and Zuazaga (1942), in serum or heparinized plasma depending upon the series of experiments under way at the time; *g*, serum or plasma chloride by the method of Volhard-Harvey (1878, 1910); and *h*, whole blood sugar by the method of Folin and Malmros (1929). Ingestion of water, salt, glucose or vitamins was as described below for individual series of experiments, and the results will be treated in four separate sections.

RESULTS. 1. Water. The most recent papers on the effects of water during work in hot climates (Lee and colleagues, 1940, 1941; Adolph, 1943; Bean and Eichna, 1943; Johnson, 1943) deal in general with the end results of dehydration. The present data show the changes which occur progressively in work at

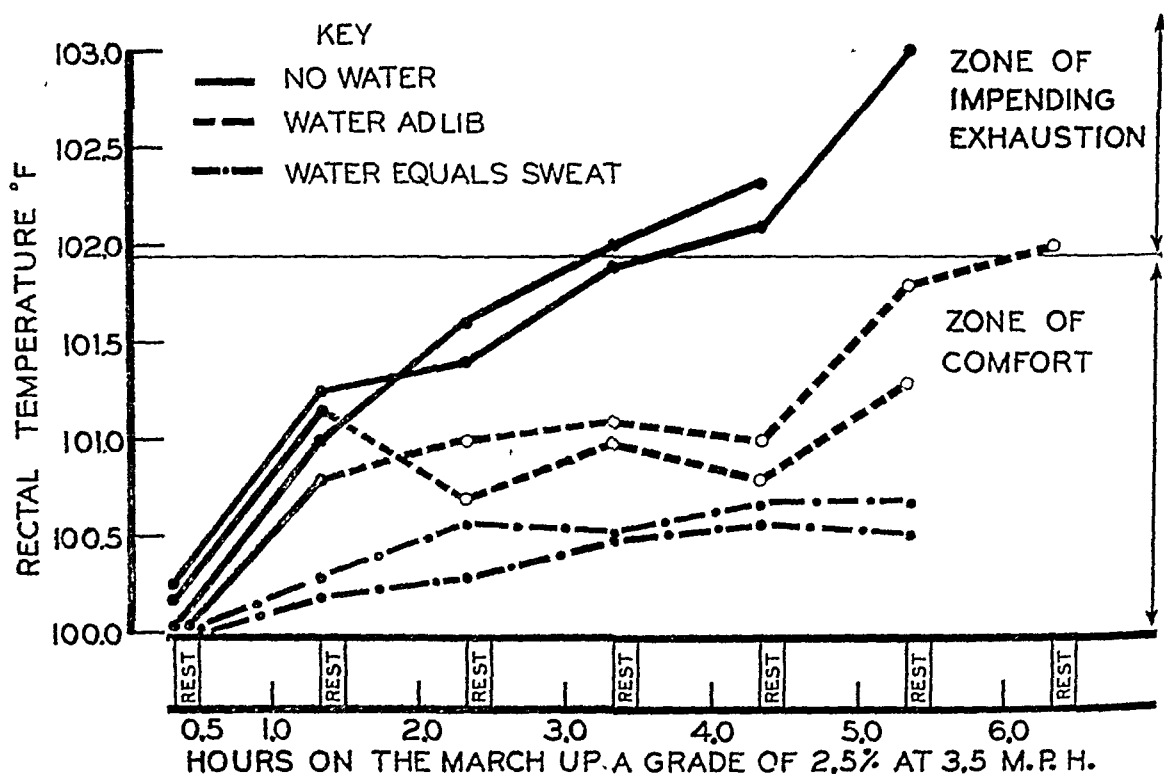


Fig. 1. Effect of water consumption on marching in the heat. (Six experiments on subject J. S. at temperature 100°F., relative humidity 35 to 45 per cent.)

various levels of water intake. Six successive experiments on a single subject marching in dry heat are illustrated in figure 1. In individual experiments he drank either nothing, water enough to keep his thirst satisfied, or water every fifteen minutes equal to the sweat lost in that period. Two complete cycles of experiments were undertaken in twelve days. Three points are clear from the figure: First, without water the rectal temperature rose steadily to high levels and showed no signs of reaching a steady state. Although the subject, being of far better than average stamina, was able to march sixteen miles, he was very tired and inefficient at the end. Second, without water for the first hour, but thereafter with enough to keep thirst quenched at all times, the rectal temperature finally started to rise after remaining constantly low for thirteen miles.

The subject was able to finish nineteen miles in fairly fresh condition. Finally, when he forced himself to drink water at the same rate as he lost sweat, the body temperature remained very low for this type of work, and the subject after sixteen miles said that he could easily go on all day. It should be emphasized that during work men never voluntarily drink as much water as they sweat,

TABLE 1

Effects of water, saline and glucose during marching in the heat (In all experiments the pace was 3.5 m.p.h., with 10 minutes' rest in each hour)

INGESTED DURING EXPERIMENT	ROUTINE MEASUREMENTS					OCCASIONAL MEASUREMENTS				
	Time of marching	Experiments averaged	Rectal temperature	Pulse rate	Rate of sweating	Experiments averaged	Oxygen consumption	Serum or plasma chloride	Serum or plasma protein	Blood sugar
A. Moist heat (dry bulb 90°F.; relative humidity, 80%; grade 4%; 2 subjects, fully clothed; constant diet)										
	hrs.	total no.	°F.	beats/min.	l./hr.	total no.	ml./kg./min.	meq./l.	gms./100 ml.	mgm./100 ml.
Nothing	1	6	101.0	136	1.48	6	21.5	107	6.3	
	3		102.4	168	1.25		23.5	108	7.4	
Water every 15 min. at rate equal $\frac{2}{3}$ sweat loss	1	6	100.6	128	1.39	6	21.6	104	6.3	
	3		100.9	142	1.29		22.3	103	7.1	
B. Moist heat (dry bulb, 95°F.; relative humidity, 83%; grade 2.5%; 5 subjects, nude)										
Nothing	1	6	101.7	143	1.17					
	2		102.7	163	0.98					
	In ten other experiments, the subjects stopped before the end of the second hour with rectal temperatures over 103°F. and pulse rates over 170									
Water every 15 min. at rate equal $\frac{2}{3}$ sweat loss	1	22	101.6	139	1.41					
	2		102.3	153	1.25					
C. Dry heat (dry bulb, 100°F.; relative humidity, 35%; grade 2.5%; 6 subjects, nude)										
Nothing	1	11	100.7	122	0.88	3	19.3	102	7.1	99
	4		102.1	154	0.76		20.0	103	8.2	112
(In all the experiments below, nothing was ingested in the first hour; water, saline or glucose were ingested every hour thereafter)										
Water ad lib.	1	7	100.9	130	0.86	1	20.7	102	7.3	
	4		101.2	143	0.74		21.2	100	7.8	
Water every 15 min. equal to sweat loss	1	6	100.8	126	0.94	2	18.8	103	7.5	104
	4		100.9	132	0.80		18.4	97	7.8	104
0.2% saline every 15 min. equal sweat loss	1	5	100.9	123	0.92	3	20.0	101	7.1	102
	4		100.9	131	0.81		20.2	99	7.6	106
No water; 25 grams glucose per hour	1	4	100.5	119	0.82	3	18.7	101	7.3	103
	4		101.5	144	0.74		19.6	101	8.1	103
Water equal sweat loss; 25 grams glucose per hour	1	3	100.6	128	0.90	3	19.2	101	7.1	98
	4		100.5	126	0.71		18.6	95	7.7	92
No water; 100 grams glucose per hour	1	1	100.7	118	0.77	1	18.6	97	7.2	95
	4		102.4	154	0.74		18.6	105	7.8	154
Water equal sweat loss; 100 grams glucose per hour	1	1	100.6	134	0.99	1	18.8	102	7.3	122
	4		101.3	148	0.77		16.9	96	8.2	160

even though this is advantageous for maintaining heat balance, but usually drink at a rate approximating about two-thirds of the water loss in sweat. Although cool water is more palatable, water at any temperature up to 100°F. is equally beneficial. The deficit is made up in the rest periods following the day's work (see Adolph and Dill, 1938; Dill, 1938).

Significant physiological and biochemical changes may occur in the heat during prolonged work without water to drink. The data presented in table 1 are most satisfactorily interpreted by comparing increments or decrements during the course of the various types of experiment. The absolute levels for any given measurement are less instructive, since the same subjects were not used in all cases, and individual peculiarities in some cases make large differences in the absolute value of the mean. However, all of the subjects showed the same type of change in individual experiments, which makes the increment or decrement more satisfactory than the absolute level for purposes of comparison. When water is withheld it is found that: the rectal temperature and pulse rate rise steadily to uncomfortably high levels; the rate of sweating declines steadily; mechanical efficiency decreases as is shown by an increase in oxygen consumption; and serum protein increases. The subject gradually feels worse and worse, and eventually becomes incapacitated from exhaustion of dehydration, no matter how tough or well acclimatized he may be. Administration of water combats all of these undesirable changes, and in general the more nearly water intake approximates sweat loss, the better off the subject remains. This is true for moist as well as dry heat as will be seen by comparing sections A and B with C in table 1.

There is a common misconception that evaporation is of relative unimportance as an avenue of heat loss under humid conditions. When men have to dissipate about 400 Cal. per hour, as our subjects did, evaporation of sweat has to be the chief source of heat loss so long as the relative humidity remains below 100 per cent. Otherwise heat exhaustion supervenes rapidly unless the rate of work decreases. Under the almost intolerable conditions listed in table 1 B, ingestion of water enabled the subjects to maintain their relative rate of sweating and strikingly to improve their performance. The reason that so few experiments without water are listed is that for comparative purposes we have listed only experiments in which the subject could finish two hours. In most of the experiments without water, the subjects failed to finish the second hour.

2. *Salt.* The ill effects of progressive depletion of the salt reserves are well known (Talbot, 1935; Taylor and colleagues, 1943). We investigated the effects of administering salt during the day's work in an attempt to determine whether this is advantageous for fully acclimatized men who receive adequate amounts of salt in the daily diet. As pointed out above (section 1) the effects of plain water are so striking that any further effects of salt have to be compared with this large effect of water. As is common experience, in experiments testing the effects of salt without water, 10 per cent saline or salt tablets without water were so distressing to the subjects that it was far preferable to go without anything. Administration of plain water equal in volume to the sweat led to the beneficial effects discussed above (table 1 C). It is appropriate to emphasize at this point that the serum chloride remains remarkably constant in prolonged work in the heat, even in spite of profuse sweating, provided the overall daily intake of salt with the meals is adequate. Therefore in the present experiments it caused no surprise that administration of 0.2 per cent aqueous saline equal in

volume to the sweat produced substantially the same effects as the administration of an equal volume of plain water. There was perhaps a tendency for the saline to maintain the rate of sweating better than plain water and also to sustain the serum chloride better. Neither of these tendencies was striking, and the performance of the subjects certainly did not benefit from them.

Further tests on this point were conducted on eleven subjects marching outdoors in a Boston summer. On two occasions all marched 10 miles continuously at 4.5 m.p.h. receiving every two miles water approximately equal in volume to the sweat. On the first occasion the first five subjects received a total of nine grams of salt in enteric coated tablets taken three grams per hour for three hours prior to the march. On the second occasion the last six subjects, but not the first five, received salt in the above manner. By comparing the averages for the two groups, it was possible to observe the effects of the salt independently of the water. There was no beneficial effect of the salt on the subjects' feelings, pulse rates or rectal temperatures. In fact, gastro-intestinal uneasiness was felt by those who had received salt tablets, and their pulse rates and rectal temperatures were slightly less good than those who received only water.

3. *Glucose.* It has been reported that ingestion of glucose may be beneficial in avoiding or treating heat cramps and heat exhaustion (see a critical review by Talbott, 1935). In testing the possible beneficial influence of glucose, we compared the effects of moderate and of large amounts of glucose with and without water to drink against the effects of water alone (table 1, C). We may dismiss the large doses (100 grams per hr.) of glucose briefly. The subjects felt nauseated and so uncomfortable that there was obviously no point in getting more data than we got in the two experiments listed. With small doses of glucose (25 grams of glucose dissolved in about 30 ml. of water) there was a small but definite advantage over experiments when no water was permitted. This could have been due to the small amounts of water in which the glucose had to be dissolved before ingestion. In these experiments with glucose, but almost no water, the increases in pulse rate and rectal temperature, and the decreases in rate of sweatings were almost as large as when neither water nor glucose were taken. The effects upon oxygen consumption, plasma chloride, plasma protein and blood sugar were insignificant. These effects were very small in comparison with the beneficial effects of water equal in volume to the sweat, and were more than counterbalanced by the feeling of gastro-intestinal uneasiness, with occasional twinges of nausea, which all of the subjects experienced. When 25 grams of glucose hourly were administered in addition to water equal to the sweat, no significant advantage in favor of glucose could be detected with the possible exception of a slightly more favorable pulse rate. There were no gastro-intestinal complaints in such experiments.

4. *Vitamins.* We have been unable to detect any advantage from administering 200 mgm. of ascorbic acid, 20 mgm. of thiamine hydrochloride, 20 mgm. of riboflavin or large doses of brewer's yeast, either singly or together either during work or the day before. In this connection, recent reports suggest that

losses of water soluble vitamins in sweat are negligible. For a review of this somewhat controversial field see Sargent, Robinson and Johnson (1944).

DISCUSSION. It would appear from the present experiments that in the case of well acclimatized young men whose daily diet is adequate, the best performance of intermittent work in the heat is to be achieved by replacing water loss hour by hour and salt loss meal by meal.

The practical limitations of this idealized situation are many. Water transport and supply are sometimes difficult. When water is available, it should not be forbidden on the traditional ground that during work it is bad for one (see Johnson, 1943, for a discussion of this point), but men should be encouraged to drink to capacity. Even the toughest, best acclimatized men suffer serious inefficiency in a few hours while working hard without water. We have seen a case of true exhaustion of dehydration in a man marching for six hours at -20°F . In addition, there is no economy in water to be gained by restriction during work, since for practical purposes the loss of sweat is about as great whether or not water is drunk.

Another practical difficulty sometimes is that a good daily diet is not available because of failure of supply or is not eaten because of ignorance, or anorexia which is so common in hot environments. Under such circumstances it is highly desirable to ensure adequate intake at least of salt, by means of salted drinking water or tablets, and probably of vitamins by means of concentrates.

SUMMARY

1. The best performance of fully acclimatized young men on a good daily diet, performing intermittent hard work in the heat, is achieved by replacing hour by hour the water lost in sweat. Any amount of water considerably less than this leads in a matter of hours to serious inefficiency and eventually to exhaustion.

2. Replacement of salt hour by hour under such circumstances has no demonstrable advantage.

3. Administration of glucose is of little if any advantage when compared with the great benefit of large amounts of water.

4. When practical problems of transportation and supply, lack of appreciation of the importance of water and salt, or the anorexia which is so common in hot environments, interfere with adequate intake, it may become desirable to supply salt in the drinking water, or less satisfactorily, in the form of tablets.

Acknowledgments. We are greatly indebted to the First Service Command and to the Quartermaster Corps, U. S. Army, for the services of T/4 J. Poulin, T/5 A. Razoyk and Pfc. J. Stachelek as technicians and subjects.

REFERENCES

- ADOLPH, E. F. Fed. Proc. 2: 15S, 1943.
ADOLPH, E. F. AND D. B. DILL. This Journal 123: 369, 1938.
BEAN, W. B. AND L. W. EICHNA. Fed. Proc. 2: 144, 1943.
DILL, D. B. Life, heat and altitude. Harvard Univ. Press, Cambridge, 1938.

- DUFOUR, L. F. *J. physiol. path. gen.* **37**: 101, 1939.
- FOLIN, O. AND H. MALMROS. *J. Biol. Chem.* **83**: 115, 1929.
- HALDANE, J. B. S. *Respiration*. Yale Univ. Press, New Haven, 1935.
- HARVEY, S. C. *Arch. Int. Med.* **6**: 12, 1910.
- JOHNSON, R. E. *Gastroenterol.* **1**: 832, 1913.
- LEE, D. H. K. AND G. P. B. BOISSARD. *Med. J. Australia* **27**: 664, 1940.
- LEE, D. H. K., R. E. MURRAY, W. J. SIMMONDS AND R. C. ATHERTON. *Med. J. Australia* **28**: 249, 1941.
- MA, T. S. AND G. ZUAZAGA. *Ind. and Eng. Chem.* **14**: 280, 1942.
- PETERS, J. P. AND D. D. VAN SLYKE. *Quantitative clinical chemistry*. Vol. II. Methods. Williams and Wilkins Co., Baltimore, 1932.
- ROBINSON, S., E. S. TURRELL, H. S. BELDING AND S. M. HORVATH. *This Journal* **140**: 168, 1943.
- SARGENT, F., P. ROBINSON AND R. E. JOHNSON. *J. Biol. Chem.* **153**: 285, 1944.
- TALBOTT, J. H. *Medicine* **14**: 323, 1935.
- TAYLOR, H. L., A. HENSCHEL, O. MICKELSEN AND A. KEYS. *This Journal* **140**: 439, 1943.
- VOLHARD, J. *Ztschr. fur Anal. Chem.* **17**: 482. 1878.

THE EFFECT OF ANEMIC ANOXIA ON THE MOTILITY OF THE SMALL AND LARGE INTESTINE¹

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Anoxic anoxia (1, 2) and anemic anoxia as well (3) have been shown to delay gastric emptying in both man and dog. It further has been demonstrated that anoxic anoxia depresses the motility and tone of the muscles of the colon of the dog (4). While the propulsive motility of the small intestine in mice has been found to be significantly depressed by moderate degrees of anoxic anoxia even severe degrees of this type of anoxia have no appreciable effect in the case of the dog.

As far as the authors are aware no quantitative studies have been reported of the effect of anemic anoxia on the motility of the intact small intestine in the unanesthetized dog, nor have studies been reported of the effect of various degrees of hemorrhage on the movements of the colon. In view of this it was deemed of interest to study the effect of anemic anoxia on the propulsive motility of the small intestine and the colon in these animals.

METHODS. a. *The effect of hemorrhage on the motility of the small intestine.* Matched pairs of animals previously fasted 24 hours were used; one as a control, the other as an experimental animal.

In one group the animals were lightly anesthetized with ether, the femoral artery exposed and a cannula inserted and an amount of blood withdrawn equal to 1.6 per cent of the body weight. The wound was closed and the animals were allowed six hours to recuperate from the effects of the anesthetic agent and the immediate effects of the hemorrhage. At the end of that time they were given 50 cc. of a suspension of 10 per cent powdered charcoal in a 10 per cent aqueous solution of gum acacia by stomach tube. Thirty minutes later a fatal concentration of ether was administered, the small intestines removed and the distance the charcoal mixture had traversed the gut measured.

In another group of dogs the same procedure was followed, except that they were hemorrhaged 3 per cent of their body weight.

In each group control animals were treated exactly the same as the experimental animals with the exception that no blood was withdrawn.

b. *The effect of hemorrhage on the motility of the colon.* Lightly barbitalized dogs were used (250 mgm. of sodium barbital per kilo body weight). The colon was exposed by a midline incision and the movements of the longitudinal muscles were recorded by means of an enterograph described by Lawson (5).

After a control tracing of the movements of the muscles of the colon was taken, a cannula was placed in the femoral vein and the animals subjected to

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successive hemorrhages as follows: 1.5, 0.7, 0.8 and 0.5 per cent of their body weight. A few of the animals withstood even more severe hemorrhage. In all instances tracings of the contraction of the colon were taken until the hemorrhage proved fatal. Although a variable interval was allowed between each bleeding, the majority of the experiments were conducted over a period of about four hours. Altogether fourteen animals were used.

RESULTS. Table 1 shows that when the animals lost an amount of blood equal to 1.6 per cent of their body weight the propulsive motility of the small intestine is not significantly influenced. The data suggest, however, that motility is somewhat accelerated. The data in table 2 show that when the animals lose blood equal to 3 per cent of their body weight, the propulsive motility of the small intestine is significantly increased.

TABLE 1

The effect of hemorrhage (1.6 per cent of body weight) on the motility of the small intestine of the dog

CONTROL				EXPERIMENTAL			
Body wt.	Length of intestine	Length of gut traversed by charcoal		Body wt.	Length of intestine	Length of gut traversed by charcoal	
kgm.	cm.	cm.	per cent	kgm.	cm.	cm.	per cent
9.78	234	152	65	10.1	201	155	77
7.88	225	168	75	7.71	260	79	30
11.6	301	157	52	8.42	270	195	72
6.29	217	98	45	6.15	317	127	40
8.28	243	126	52	11.0	317	258	81
9.67	268	229	85	7.71	323	213	66
16.2	319	245	77	12.2	323	272	84
10.4	235	62	26	8.84	225	196	87
16.3	319	250	78	10.4	260	182	70
11.1	322	47	15	12.8	327	205	63
Avg....10.8	268	153	57*	9.53	282	188	67*

* Difference = 10 per cent; $t = 1.0629$; P (according to Fisher) = $>.20$.

Even though pairs of dogs matched in weight and size were used, there was considerable individual variation in the total length of the gut. It was necessary, therefore, to use percentage figures; these have an elliptical distribution. Since this type of data does not lend itself accurately to statistical treatment unless all the values fall above 10 and below 90 per cent, only those data which fell within these limits were used.

The accompanying figures (1-3) illustrate the effect of graded hemorrhages on the motility and tonus of the longitudinal muscles of the colon. Whereas they all show that hemorrhage is capable of producing a depression of colonic motility, each graph illustrates a specific feature. Figure 1 shows a marked reduction in the height of contractions as well as a decrease in number following hemorrhage. Figure 2 illustrates that hemorrhage is capable of producing a

noticeable reduction in tone of the muscles of the colon. Figure 3 shows that the muscles of the colon are still capable of contracting even though the animal has suffered a severe hemorrhage.

TABLE 2
The effect of hemorrhage (3 per cent of body weight) on the motility of the small intestine of the dog

CONTROL				EXPERIMENTAL			
Body wt.	Length of intestine	Length of gut traversed by charcoal		Body wt.	Length of intestine	Length of gut traversed by charcoal	
kgm.	cm.	cm.	per cent	kgm.	cm.	cm.	per cent
10.8	259	195	75	11.7	263	170	65
7.71	207	88	43	8.05	200	174	87
8.33	261	63	24	7.43	186	161	87
6.24	267	147	55	7.20	204	160	78
10.7	327	254	78	10.8	333	200	60
13.2	289	115	40	13.6	242	150	62
7.96	237	123	52	8.11	312	256	82
14.1	265	240	91	14.6	271	246	91
				12.64	300	138	46
6.18	236	79	33	5.44	181	140	77
Avg.... 9.47	261	145	55*	8.64	249	180	74*

* Difference = 19 per cent; $t = 2.302$; P (according to Fisher) ≈ 0.033 .

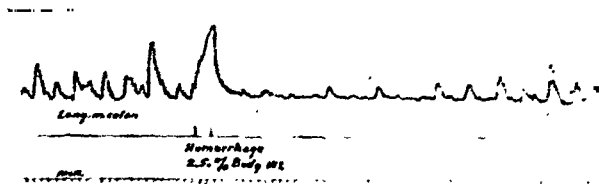


Fig. 1

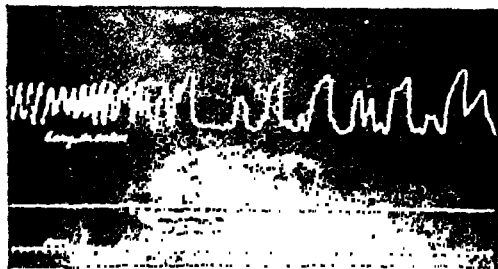


Fig. 2

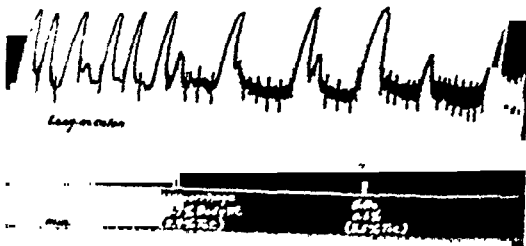


Fig. 3

DISCUSSION. It is difficult to explain why hemorrhage increases the propulsive motility of the small intestine for it definitely has been shown (3) that gastric emptying is delayed by hemorrhage both in man and in the dog.

It is known that hemorrhage produces changes in the organism which simulate those produced by stimulation of the sympathetic division of the autonomic

nervous system. Following a significant hemorrhage the heart rate increases, vasoconstriction occurs, gastric motility is decreased and certain other changes occur which somewhat simulate sympathetic stimulation. Since the innervation of the small intestine presumably is the same as that of the stomach, it would be expected that hemorrhage likewise would have a depressing effect on gut motility.

It has been shown in our laboratory that the small intestine of the dog is extremely resistant to anoxic anoxia (4). Even severe degrees of anoxia (partial pressures of O_2 from 80–43 mm. Hg) had no significant depressing action on its propulsive motility. It now appears that the small intestine is quite resistant to anemic anoxia as well.

It has been shown by Gellhorn and his co-workers (6) that anoxic anoxia is capable of stimulating both sympathetic and parasympathetic centers. As a rule sympathetic stimulation predominates and masks the parasympathetic effect. Gellhorn and his associates have further shown that the vago-insulin system and sympathetico-adrenal system may be stimulated as well by such agents as cold and heat (7) and still others.

To our knowledge it has never been shown that hemorrhage (anemic anoxia) causes a greater stimulation of the parasympathetic nerves than of the sympathetic. Our results, however, appear to indicate that hemorrhage produced a greater stimulation of the parasympathetic nerves which supply the small intestine than it did the sympathetic; for the present this seems to be the most plausible interpretation.

It is possible that a humoral mechanism may be involved. In 1918 Carlson (8) reported experiments performed on the effect of acute hemorrhage on hunger contractions. He observed that after dogs had been bled 30 per cent of their calculated blood volume, the tone of the stomach was increased and hunger contractions intensified. This augmented effect disappeared in less than twenty-four hours. His interpretation was that tissues deprived of so much nutritive material probably liberated a hormone which stimulated hunger contractions. This hypothesis, for which no subsequent proof has been offered, is an inviting one and would explain why hemorrhage caused an increase in propulsive motility of the small intestine.

It is in order now to discuss briefly the effect of hemorrhage on the motility of the colon. The graphs indicate that hemorrhage may produce a decrease in the height or number of the contractions of the colon or a change in tone or some combination of these factors. The general effect is one of depression.

That hemorrhage may initially stimulate the colonic musculature is evident in figure 1. A number of times it was observed that immediately following a hemorrhage the longitudinal muscles of the colon showed a distinct spasm, later followed by a depression of activity. The intestinal spasm was not considered a result of mechanical manipulation, since every effort was made to minimize this factor; moreover, the animal was under light barbital anesthesia. The marked dyspnea produced by the anemic anoxia is clearly indicated in figure 3.

Of the fourteen animals used the majority showed some depression of colonic activity following a hemorrhage equal approximately to 1.5 per cent of their

body weight. This was considered the threshold for the average animal. Two of the fourteen animals were very resistant to hemorrhage and the activity of the colon showed but little depression until the amount of blood withdrawn caused the animal to cease breathing.

On the whole, the experiments indicate that the activity of the longitudinal muscles of the colon are relatively resistant to anemic anoxia. It should be borne in mind, however, that the dog has a very short colon and the results obtained in this animal may not be entirely applicable to man.

SUMMARY

A study was made of the effect of hemorrhage (anemic anoxia) on the motility of the colon in lightly barbitalized dogs and of the small intestine in unanesthetized animals.

Following a significant hemorrhage the longitudinal muscles of the colon, as recorded by an enterograph, may show a decrease in the height or in the number of contractions, a change in tone or some combination of these factors. A few of the animals studied were highly resistant to the effects of hemorrhage; however, the majority showed a depression in activity of colonic musculature after they had lost a quantity of blood equal to 1.5 per cent of their body weight.

In a group of dogs subjected to a hemorrhage equivalent to 3 per cent of their body weight, it was observed that a powdered charcoal-acacia mixture, given by stomach tube had traversed 74 per cent of the total length of the small intestine at the end of thirty minutes. In contrast, the value for the control group was 55 per cent. The difference was highly significant statistically. No entirely adequate explanation can be offered why anemic anoxia accelerated the propulsive movements of the small intestine. The most plausible explanation in this instance is that, contrary to the general rule, hemorrhage stimulated the parasympathetic nerves more than it did the sympathetic.

We wish to express our thanks to Dr. Hampden Lawson for the use of his enterograph.

REFERENCES

- (1) VAN LIERE, E. J., D. H. LOUGH AND C. K. SLEETH. *Arch. Int. Med.* 58: 130, 1936.
- (2) VAN LIERE, E. J., G. CRISLER AND D. ROBINSON. *Arch. Int. Med.* 51: 796, 1933.
- (3) VAN LIERE, E. J., C. K. SLEETH AND D. W. NORTHUP. *This Journal* 117: 226, 1936.
- (4) VAN LIERE, E. J., D. W. NORTHUP, J. C. STICKNEY AND G. A. EMERSON. *This Journal* 140: 119, 1943.
- (5) LAWSON, H. *J. Lab. Clin. Med.* 20: 496, 1935.
- (6) FELDMAN, J., R. CORTELL AND E. GELLHORN. *This Journal* 131: 281, 1940.
- (7) GELLHORN, E. AND J. FELDMAN. *This Journal* 133: 670, 1941.
- (8) CARLSON, A. J. *The control of hunger in health and disease.* Univ. of Chicago Press, 1916, Chicago.

THE EFFECT OF VITAMINS OF THE B COMPLEX ON THE WORK OUTPUT OF PERFUSED FROG MUSCLES

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In view of the important rôle played by vitamins of the B group in the enzyme systems associated with energy transformations in the animal body (29) it is reasonable to expect some relationship between dietary deficiencies in these vitamins and work output. Johnson et al. (18) have reported complaints of easy fatigue and deterioration of physical fitness within a period of 7 to 10 days in previously normal adults subjected to hard physical labor when maintained on thiamine deficient diets. Barborka et al. (4) also found a diminution in work output in trained subjects when the intake of vitamin B complex was lowered. Work output increased to previous levels when the vitamin B complex was restored to the diet. Increased fatigability was reported by Williams, Mason et al. (36) in women subjected to only slight reduction in vitamin B₁ intake. Keys (20, 21, 22) on the basis of tests made on young adult males categorically denies an early reduction in work output as a result of vitamin B complex deficiency. Kniazuk and Molitor (23) have shown that in rats a thiamine deficient diet results in a rapid and marked decrease in work-performance which is promptly restored by the administration of thiamine. The work-performance of nondeficient animals was not significantly improved by the administration of large doses of thiamine. Excessive intake of vitamins has not proved effective in increasing the work output in human subjects receiving an adequate diet (12, 21, 22, 32), although administration of surplus vitamin B complex has been reported to increase the fusion frequency of flicker (32). Nicotinic acid amide administration has also been reported to increase the efficiency of adequately nourished young adults in performing tasks requiring both physical effort and co-ordination (13). Much of this literature has been reviewed recently by Keys (20).

Thus there is considerable disagreement and confusion in the experimental data on humans with respect to the effect of vitamins on muscular work. Part of this disagreement may lie in the difficulties of measuring "fatigue", "work output", or "physical fitness" in the human subject. Nor can it be said that all experiments reported were free from psychological and training factors which may greatly influence the work output of humans. In animal experiments certain of these disturbing factors may be minimized and even eliminated. In such experiments uniform conditions of stimulation can be maintained and a fatigue curve for a single muscle can be obtained under standardized conditions. Known concentrations of pure vitamin products can be perfused through the muscle

¹ With the technical assistance of Marvin J. Yiengst.

and fatigue curves obtained from which the work done can be estimated. With this aim the following experiments were performed on perfused frog preparations. The effect of thiamine, riboflavin, nicotinic acid amide, pyridoxine and calcium pantothenate on work output was tested on perfused frog muscles. It should be noted that results from such animal experiments cannot be directly applied to the work capacity of the intact organism since reduction of work output in the intact organism is rarely due to fatigue of the muscle itself.

EXPERIMENTAL. The experimental procedure was adapted from that of Eddy (10). The fatigue curve was graphically recorded for each gastrocnemius muscle of a frog, uniformly loaded, and uniformly stimulated. One gastrocnemius was perfused through the general circulation with a buffered glucose-Ringer's solution only, the other was perfused in like manner with the vitamin added to the Ringer's solution. Medium sized summer frogs (*Rana pipiens*) were used in the experiments. The frog was pithed and the spinal cord destroyed. Fine copper wires (no. 36) were attached to each Achilles tendon and a cannula was inserted into the aorta through a cut in the ventricle. A buffered glucose-Ringer's solution was perfused through the animal at a rate of 3.0 cc. per minute. The rate was controlled by a stopcock and was kept uniform throughout the experiment. The auricles were cut to permit free outflow of the perfusion fluid. The frog was mounted vertically on a board and the wire from the tendon attached to an isotonic muscle lever recording on a smoked drum with a magnification of 1 to 9.5. A ten gram load was attached directly to the muscle which was not after-loaded. Stimulation was afforded by break shocks (make shocks shorted) from the inductorium through the copper wire attached to the tendon and a needle inserted into the knee joint. Stimuli eliciting initially maximal contractions were applied automatically through a motor driven commutator at a rate of 32 per minute. The primary current to the inductorium was broken by a spring relay with silver contacts.

The buffered glucose-Ringer's solution used had the following composition: 27.4 mM NaHCO_3 per liter, 2.0 mM KCl per liter, 86.0 mM NaCl per liter, 1.1 mM CaCl_2 per liter, 2.0 grams dextrose per liter and 7.5 mgm. phenol red per liter. A stock solution containing the NaHCO_3 , KCl , NaCl and phenol red was prepared and kept saturated with a gas mixture of 5 per cent CO_2 -95 per cent O_2 . Glucose and CaCl_2 were added to the stock solution just before use. The calcium content of the perfusion fluid was adjusted to 1.1 mM per liter by diluting 12.1 ml. of a 90.8 mM/l. stock solution of CaCl_2 to 1 liter with the glucose-Ringer's solution that had just been prepared. The Ca content of the standard CaCl_2 solution was determined manometrically by the method of Van Slyke and Sendroy (27). Any solutions from which CaCO_3 began to precipitate were discarded. The pH of the buffered perfusion fluid was 7.40 ± 0.05 at 25°C . as calculated and checked with the glass electrode. A mixture of 5 per cent CO_2 -95 per cent O_2 (checked by analysis on a 10.0 cc. Haldane gas apparatus) was continuously bubbled through the perfusion fluid during the experiments.

Stock solutions containing 100 mM per liter of each of the vitamins were prepared by weighing the appropriate amount of the pure vitamin and dis-

solving in distilled water. These stock solutions were kept in the refrigerator and 1.0 ml. of a 1 to 100 dilution added to each 100 ml. of buffered glucose-Ringer's just prior to use to give a final concentration of 0.01 mM per liter of the vitamin in the perfusion fluid.

After preparation of the animal a fatigue curve was run on the left gastrocnemius muscle while perfused with the vitamin-free glucose-Ringer's solution under the conditions outlined. Stimulation was continued for 15 minutes. At the end of this time the right gastrocnemius muscle was attached to the muscle lever recording system and a single break shock administered to see that its initial contraction was not greatly different from that obtained originally from the left gastrocnemius. If the contractions were similar, the perfusion fluid was changed to that containing the vitamin to be tested. The perfusion was continued for 15 minutes during which time the right muscle was after-loaded to prevent stretching. At the end of this time the after-loading was removed and a fatigue curve was recorded with stimulation for 15 minutes. Methylene blue was then added to the perfusion fluid to test the adequacy of the perfusion. All experiments in which there was any difference in the intensity of the blue color of the two legs, or difference in the rate at which the color developed in the two legs, were discarded. Groups of 10 animals were used in testing each vitamin.

Treatment of the data. From the fatigue curves, examples of which are shown in figure 1, A and B, the total work done by each muscle was estimated in the following manner. The height of contraction was measured at each minute of the exercise. These values were plotted on co-ordinate paper and a smooth curve drawn through the points as shown in figure 2. The area bounded by this smooth curve, the 15 minute ordinate and the x and y axes, was measured with a planimeter. This area, multiplied by an appropriate factor² gave the total work done in gram-centimeters.

Average values of work done, with their standard errors, were computed for each set of 10 experiments and the differences between the work done by the

² Let y = the height of each contraction recorded on the drum and k_1 = magnification of lever arm (1/9.5)

Work done per contraction = $k_1 y m$, where m = weight lifted.

Work done in time dt is therefore,

$$dW = k_1 y m dt \text{ and the total work done between } t_1 \text{ and } t_2$$

with a stimulation rate of 32 per minute under the conditions of the present experiment is,

$$\begin{aligned} W &= 32 k_1 m \int_{t_1}^{t_2} y dt \\ &= 32 \frac{1}{9.5} 10 \int_{t_1}^{t_2} y dt = 33.7A \end{aligned}$$

where $A = \int_{t_1}^{t_2} y dt$ = Area beneath curve in millimeter minutes.

control and experimental muscles were calculated.³ Statistical tests of the significance of the observed differences were applied.

RESULTS. Total work output. Experiments were first run in which the buffered Ringer's solution was perfused through both legs. Fatigue curves from such an experiment are shown in the upper part of figure 1 (A). Similar performance was observed in each leg. In a series of 10 experiments the average work output of the first leg (left) was 1891 ± 82.2 gram-centimeters⁴ while that of the second leg (right) was 1822.6 ± 82.1 gram-centimeters. The difference of 68.6 gram-centimeters was not statistically significant (see line 1, table 1).

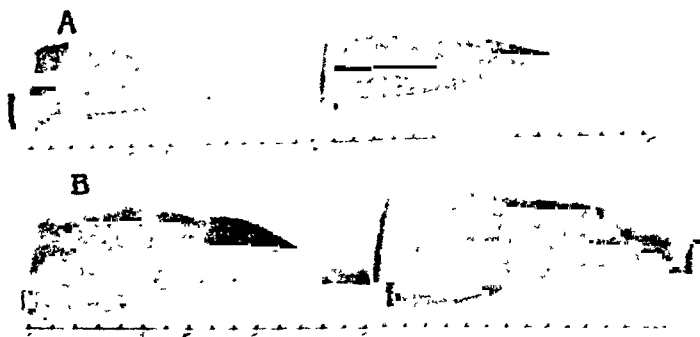


Fig. 1. Work curves of frog gastrocnemius muscles. Isotonic muscle lever, 9.5:1 magnification. 10 gram load, no afterloading. Stimulation 32 per min., break shocks. A. Left and right muscles perfused through general circulation with buffered glucose-Ringer's solution. B. Left muscle perfused with buffered glucose-Ringer's solution; right muscle, perfused with buffered glucose-Ringer's plus 0.01 mM thiamine hydrochloride per L.

The lower part of figure 1 (B) shows sample fatigue curves obtained when 0.01 mM thiamine was added to each liter of perfusion fluid. Average values of total work output obtained when 0.01 mM per liter of the various vitamin preparations were added to the buffered glucose-Ringer's perfused through the

For the planimeter used, 1000 mm. min. = 1.550 units

$$f = \frac{33.7 \cdot 50}{1.550} = 1085$$

Although these measurements do not give the absolute magnitude of the work done because of differences in muscle viscosity, inertia of the lever, etc., the values obtained are closely correlated with the true work of the muscle.

³The standard error of the mean was calculated from the formula $\sigma_{M_n} = \frac{\sigma_d}{\sqrt{N}}$ where σ_d is the standard deviation of the distribution calculated as $\sqrt{\frac{\sum d^2}{N-1}}$. The standard error of the difference between the means was calculated from the formula

$$\sigma_{M_{n_x} - M_{n_y}} = \sqrt{\sigma_{M_{n_x}}^2 + \sigma_{M_{n_y}}^2 - 2r_{xy}\sigma_{M_{n_x}}\sigma_{M_{n_y}}}$$

where

$$r_{xy} = \frac{\sum xy}{N\sigma_x\sigma_y} \text{ (Guilford, 15)}$$

⁴All means are followed by ± 1 standard error.

second leg are assembled in table 1. This table shows that addition of either calcium pantothenate or thiamine hydrochloride at a concentration of 0.01 mM per liter to the perfusion fluid results in an increase in work output of about 18 per cent which is statistically significant. The increase in work output observed with pyridoxine and nicotinic acid amide is not statistically significant.⁵ Riboflavin, at a concentration of 0.01 mM per liter had no apparent effect on the total work output.

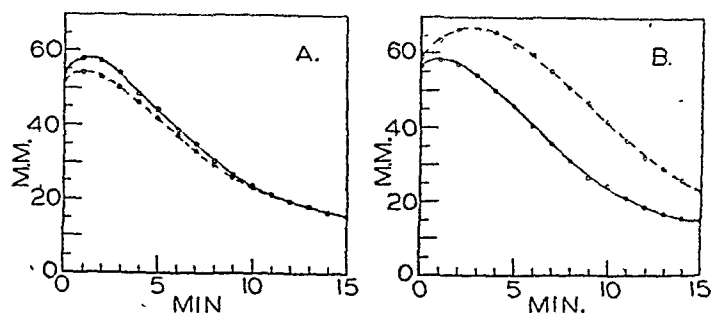


Fig. 2. Plots of work curves of frog gastrocnemius muscles, showing height of contraction. Left muscle——; Right muscle———. A. Right and left muscles perfused with buffered glucose-Ringer's solution. B. Left muscle perfused with buffered glucose-Ringer's solution, right muscle perfused with glucose-Ringer's solution plus 0.01 mM/L thiamine.

TABLE 1

Effect of vitamin administration on the total work output of perfused frog muscle

	WORK OUTPUT CONTROL LEG	WORK OUTPUT, EXPERIMENT LEG	r_{xy}	AV. INCREASE IN WORK DONE	CRITICAL RATIO
	gm.-cm.	gm.-cm.		gm.-cm.	
Control.....	1891.2 \pm 82.2*	1822.6 \pm 82.1*	0.73	68.6 \pm 60.4*	1.1
Ca pantothenate 0.01 mM/l.....	1945.8 \pm 55.6	2304.3 \pm 71.8	0.45	358.5 \pm 68.2	5.2
Thiamine HCl 0.01 mM/l.....	1952.4 \pm 72.6	2219.1 \pm 61.2	0.41	366.7 \pm 73.3	5.0
Pyridoxine HCl 0.01 mM/l.....	1794.5 \pm 54.2	1961.2 \pm 58.5	0.48	166.7 \pm 57.6	2.9
Nicotinamide 0.01 mM/l.	1896.0 \pm 43.3	2011.0 \pm 59.1	0.42	115.0 \pm 56.7	2.0
Riboflavin† 0.01 mM/l...	1967.0 \pm 122.2	1953.2 \pm 135.8	0.65	-13.8 \pm 108.1	0.1

* All means are followed by ± 1 standard error.

† Based on 6 experiments; in all others N = 10.

Distribution of work output. With the above demonstration of an increase in total work output with the perfusion of thiamine or calcium pantothenate, an analysis of the effects of vitamin administration on the distribution of work during the exercise period was made. For this purpose the average height of

⁵ It may be noted that if a single aberrant experiment is excluded from the pyridoxine series the results show a mean difference of 202.2 ± 48.1 gram centimeters of work which has a critical ratio of 4.2. Similar exclusion of one experiment from the nicotinic acid amide series increases the mean difference to 196.4 ± 46.0 gram centimeters with a critical ratio of 4.2.

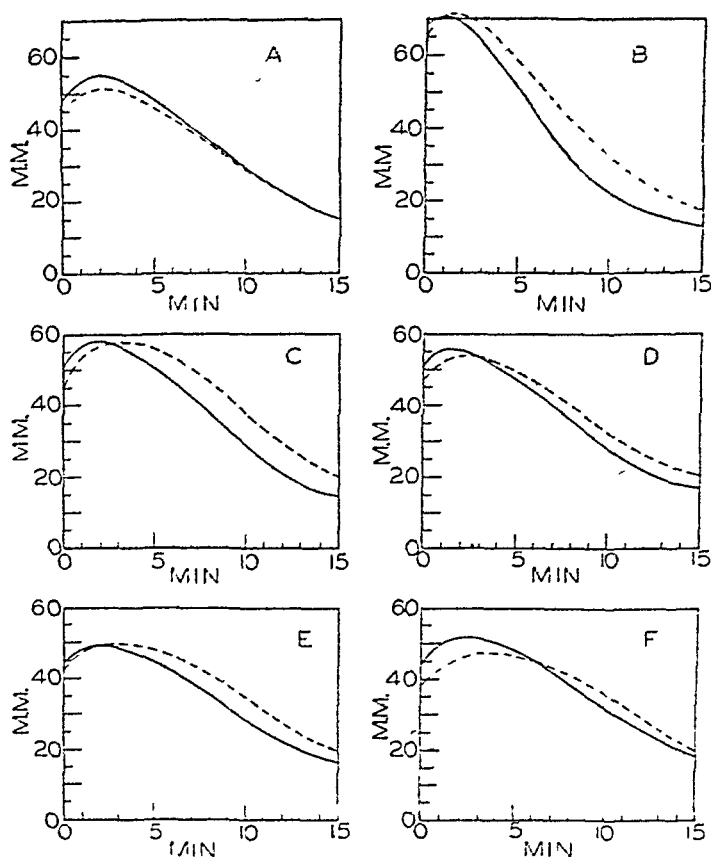


Fig. 3. Average work curves for frog gastrocnemius muscles. Left muscle perfused with buffered glucose-Ringer's solution; —. Right muscle perfused with buffered glucose Ringer's solution plus the vitamin preparation; ----. A. Control—no vitamin. B.—0.01 mM/L Calcium pantothenate; C.—0.01 mM/L Thiamine hydrochloride; D 0.01 mM/L Nicotinic acid amide; E, 0.01 mM/L pyridoxine hydrochloride; F, 0.01 mM/L riboflavin.

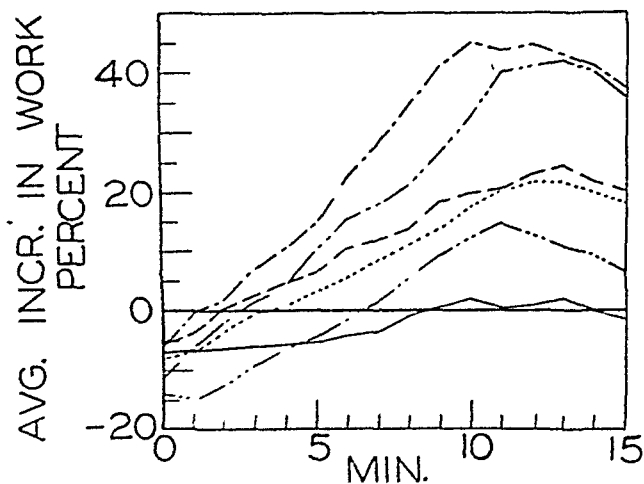


Fig. 4. Average percent increase in work of perfused frog gastrocnemius muscle — Control (no vitamin added to perfusion fluid of second muscle) ---- Ca pantothenate ---- Thiamine hydrochloride. ---- Pyridoxine hydrochloride ---- Nicotinic acid amide. ---- Riboflavin. Differences greater than 18 percent are statistically significant. Negative values indicate smaller average contraction of the second or vitamin treated muscle than of the untreated muscle.

contraction at each minute of the work period was calculated for the control and vitamin treated muscles. Plots of these average curves are shown in figure 3. The average difference between the control and vitamin treated muscle at each minute was calculated. Figure 4 shows a plot of the percentage differences.⁶ The standard error of the mean difference was calculated for the point showing the maximum difference (in mm.) with the following results; calcium pantothenate 10.8 ± 1.97 mm. (C.R. = 5.5) at 9 min.; thiamine, 9.5 ± 2.66 mm. (C.R. = 3.6) at 11 min.; nicotinic acid amide, 5.0 ± 1.66 mm. (C.R. = 3.0) at 11 min.; pyridoxine 5.7 ± 1.72 mm. (C.R. = 3.3) at 12 min. and riboflavin 4.3 ± 3.51 mm. (C.R. = 1.2) at 11 min. Thus the improvement is statistically significant for all the vitamins tested except riboflavin. Figure 4 shows that addition of all the vitamins tested increases the work output most markedly in the later stages of fatigue. In fact, no improvement is observed during the first two or three minutes of exercise. While the total work output was not significantly increased by pyridoxine or nicotinic acid amide, the later performance is significantly improved by these two vitamins as well as by thiamine and calcium pantothenate. The observed rise in the riboflavin curve is not statistically significant.

DISCUSSION. The total work obtained from the perfused frog muscles under the conditions of these experiments is slightly greater than that reported by Eddy (1136 to 1867 gram-centimeters).⁷ This increase may be due to the addition of glucose and oxygen to the perfusion fluids. The work output is of the same order of magnitude in both experiments.

Search of the literature failed to reveal values for vitamin levels in frog blood. Even values for blood levels of these vitamins in mammals are not always in agreement. However, such variations are usually less than 100 per cent, so we may assume that the order of magnitude is approximately 10 micrograms per 100 cc. for thiamine (5, 14, 16, 25), 750 micrograms per 100 cc. for nicotinic acid amide (9, 17, 24); 25 micrograms per 100 cc. for pantothenic acid (27, 30, 34, 37); and 50 micrograms per 100 cc. for riboflavin (3, 35). No values for blood levels of pyridoxine were found. When calculated in terms of mM per liter of blood the concentrations are 0.0003 mM thiamine per liter, 0.06 mM nicotinic acid amide⁸ per liter, 0.001 mM pantothenic acid per liter and 0.001 mM riboflavin per liter. Thus, with the exception of thiamine, the concentrations of vitamin present in the perfusion fluid used in our experiments was about ten times greater than that normally found in mammalian plasma. This level is still probably within nontoxic limits, since a tenfold or more increase in pantothenic acid,

⁶ Calculated as

$$\frac{\text{Mean difference}}{\text{Mn height of control curve}} \times 100$$

⁷ Comparisons between the two studies are not strictly valid since the rate of stimulation was 1 per 5 sec. and exercise was continued for 19.5 to 30.5 min. in Eddy's experiments. Both factors should tend to increase the total amount of work performed in his experiments.

⁸ This value is for whole blood. Since most of the nicotinic acid is combined in the red cells, plasma values are much lower, probably less than 0.003 mM per liter.

riboflavin (31) and nicotinic acid (2, 11) has been produced experimentally in animals and man without untoward effects. Further experiments are required to determine the most effective concentrations of these vitamins in the perfusion fluid for increasing work output. It is quite possible that in appropriate concentrations, the effect of pyridoxine and nicotinic acid amide may be shown to be significant.

Briem (8) has reported an increase in the work output of frog muscle when vitamin B₁ was injected into the lymph sac of the intact frog. However, with isolated muscles, the contractility was decreased. Unfortunately no information is given which permits an estimate of the concentrations of thiamine attained in the circulating blood. Some experiments have been performed on the effect of thiamine on the isolated frog heart, but the results are inconclusive, since buffered solutions were not always used. In general, low concentrations of the vitamin increased the strength of the heart beat, while high concentrations caused arrest, probably owing to the acidity and hypertonicity of the solutions used (6, 19).

Speculation as to the mechanism of the observed effect does not seem to be fruitful at the present state of our knowledge. However, it is highly improbable that the effects are due to vasomotor changes, with resultant changes in flow through the muscles. Previous reports have indicated a slight vaso-constrictor effect of thiamine in the frog (7). While the vaso-dilating effect of nicotinic acid is well known (1, 33) no such effect is observed with the amide, which was used in the present study (1). In all of our experiments the perfusion rate was kept constant. Thus it does not seem probable that the effects observed can be attributed to changes in the rate of flow through the muscle.

CONCLUSIONS

The effect on the work output of the frog gastrocnemius muscle by the separate addition to the perfusion fluid of 0.01 mM per liter thiamine, nicotinic acid amide, calcium pantothenate, riboflavin and pyridoxine was determined.

1. The total work output was significantly increased by thiamine and by calcium pantothenate.

2. An increased total work output was observed with nicotinic acid amide and pyridoxine, but the increase was not statistically significant.

3. A significantly greater work output was observed during the final stages of fatigue with the addition of nicotinic acid amide and pyridoxine as well as with thiamine and calcium pantothenate.

4. No increase in work output was observed with riboflavin.

REFERENCES

- (1) ARING, C. D., H. W. RYDER, E. ROSEMAN, M. ROSENBAUM AND E. B. FERRIS. *Arch Neurol. and Psychiat.* 46: 649, 1941.
- (2) AXELROD, A. E., E. S. GORDON AND C. A. ELVEHJEM. *Am. J. Med. Sci.* 199: 697, 1940.
- (3) AXELROD, A. E., T. D. SPIES AND C. A. ELVEHJEM. *Proc. Soc. Exper. Biol. [and Med.]* 46: 146, 1941.
- (4) BARBORKA, C. J., E. E. FOLTZ AND A. C. IVY. *J. A. M. A.* 122: 717, 1943.

- (5) BENSON, R. A., C. M. WITZBERGER, L. B. SLOBODY AND L. LEWIS. *J. Pediat.* **21**: 659, 1942.
- (6) BOYD, E. M. AND R. W. DINGWALL. *Quart. J. Pharm. and Pharmacol.* **14**: 209, 1941.
- (7) BRECHT, K. *Pflüger's Arch.* **243**: 714, 1940.
- (8) BRIEM, H. J. *Pflüger's Arch.* **242**: 450, 1939.
- (9) DORFMAN, A., S. A. KOSER, M. K. HORWITT, S. BERKMAN AND R. SAUNDERS. *Proc. Soc. Exper. Biol. and Med.* **43**: 434, 1940.
- (10) EDDY, N. B. *This Journal* **69**: 432, 1924.
- (11) FIELD, H., JR., D. MELNICK, W. D. ROBINSON AND C. F. WILKINSON, JR. *J. Clin. Investigation* **20**: 379, 1941.
- (12) FOLTZ, E. E., A. C. IVY AND C. J. BARBORKA. *J. Lab. and Clin. Med.* **27**: 1396, 1942.
- (13) FRANKAU, I. M. *Brit. Med. J.* **2**: 601, 1943.
- (14) GOODHART, R. *J. Clin. Investigation* **20**: 625, 1941.
- (15) GUILFORD, J. P. *Psychometric methods*. McGraw Hill, New York, 1936, 566 pp.
- (16) HENNESSY, D. J. AND L. R. CERECEDO. *J. Am. Chem. Soc.* **61**: 179, 1939.
- (17) ISBELL, H., J. G. WOOLLEY, R. E. BUTLER AND W. H. SEBRELL. *J. Biol. Chem.* **139**: 499, 1941.
- (18) JOHNSON, R. E., R. C. DARLING, W. H. FORBES, L. BROUHA, E. EGANA AND A. GRABIEL. *J. Nutrition* **24**: 585, 1942.
- (19) KAISER, P. *Pflüger's Arch.* **242**: 504, 1939.
- (20) KEYS, A. *Fed. Proc.* **2**: 164, 1943.
- (21) KEYS, A. AND A. F. HENSCHEL. *J. Nutrition* **23**: 259, 1942.
- (22) KEYS, A., A. F. HENSCHEL, O. NICKELSEN AND J. M. BROZEK. *J. Nutrition* **26**: 399, 1943.
- (23) KNIAZUK, M. AND H. MOLITOR. *J. Pharmacol. and Exper. Therap.* **80**: 362, 1944.
- (24) MELNICK, D., W. D. ROBINSON AND H. FIELD, JR. *J. Biol. Chem.* **136**: 157, 1940.
- (25) MEIKELJOHN, A. P. *Biochem. J.* **31**: 1441, 1938.
- (26) PEARSON, P. B. *J. Biol. Chem.* **140**: 423, 1941.
- (27) PEARSON, P. B. *This Journal* **135**: 69, 1941.
- (28) PETERS, J. P. AND D. D. VAN SLYKE. *Quantitative clinical chemistry*. Williams & Wilkins Co., Baltimore, 1932, vol. 2, p. 421.
- (29) POTTER, V. R. *J. Dietetic Assn.* **19**: 488, 1943.
- (30) SIEGEL, L., T. J. PUTNAM AND J. C. LYNN. *Proc. Soc. Exper. Biol. and Med.* **47**: 362, 1941.
- (31) SILBER, R. H. AND K. UNNA. *J. Biol. Chem.* **142**: 623, 1942.
- (32) SIMONSON, E., N. ENZER, A. BAER AND R. BRAUN. *J. Ind. Hyg. and Toxicol.* **24**: 83, 1942.
- (33) SPIES, T. D., W. B. BEAN AND R. E. STONE. *J. A. M. A.* **111**: 582, 1938.
- (34) STANBERRY, S. R., E. E. SNELL AND T. D. SPIES. *J. Biol. Chem.* **135**: 353, 1940.
- (35) STRONG, F. M., R. E. FLENEY, B. MOORE AND H. T. PARSONS. *J. Biol. Chem.* **137**: 363, 1941.
- (36) WILLIAMS, R. D., H. L. MASON, B. F. SMITH AND R. M. WILDER. *Arch. Int. Med.* **69**: 721, 1942.
- (37) WRIGHT, L. D. *J. Biol. Chem.* **147**: 261, 1943.

THE EFFECT OF CHANGES IN CONCENTRATION OF PANTOTHENATE ON THE WORK OUTPUT OF PERFUSED FROG MUSCLES¹

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The addition of calcium pantothenate at a concentration of 0.01 mM per liter has been shown to increase significantly the work output of perfused frog muscles (3). In the present report data on the relation between the concentration of calcium or sodium pantothenate in the perfusing fluid and the increase in work output are presented.

EXPERIMENTAL. The experimental methods used have been described in detail in a previous report (3). Summarized briefly, fatigue curves were recorded for each gastrocnemius muscle of the frog under comparable conditions of perfusion, stimulation, loading and recording. Calcium pantothenate was added to the buffered glucose-Ringer's solution used for perfusion of the frog while the fatigue curve was recorded on the second gastrocnemius. Ten animals were tested with each concentration of calcium pantothenate. Calcium pantothenate was added to the perfusion fluid in final concentrations of 0.001, 0.003, 0.005, 0.0075, 0.01, 0.5, 0.75 and 1.0 milliequivalent per liter. When concentrations of 0.5, 0.75 and 1.0 milliequivalent of calcium pantothenate were used, the total Ca concentration was kept at 1.1 mM per liter by reducing the amount of CaCl_2 added to the glucose-Ringer's by the appropriate amount (3). The concentration of the stock solution of 50 mM calcium pantothenate was checked by analysis for Ca (2) and for N by micro Kjeldahl analysis (2)². The hygroscopic nature of sodium pantothenate made it necessary to determine the concentration of the stock solution of this salt by micro-Kjeldahl analysis alone (2)³.

RESULTS. *Effect of increasing concentration of calcium pantothenate on work output.* As the concentration of calcium pantothenate was increased from 0.001 to 0.01 milli equivalent per liter in the perfusion fluid, the total work output of the treated muscle increased from a statistically insignificant difference to as much as 18 per cent above the control values. As may be seen from figure 1-A, the increase in work output appears linear from the available data. Raising the concentration of calcium pantothenate to 0.5 milliequivalent per liter increased the work output only slightly above that obtained with 0.01 milliequivalent. Figure 1-B shows that pantothenate concentrations of 0.75 or 1.0 milliequivalent

¹ With the technical assistance of Marvin J. Yiengst.

² On a sample weighed as 50.0 mM calcium pantothenate per liter, analysis for Ca gave a value of 49.85 mM per liter; micro-Kjeldahl analysis of the same solution gave a value of 49.35 mM calcium pantothenate per liter.

³ The stock solution contained 66.8 mM sodium pantothenate per liter.

per liter failed to increase the physiological response beyond that observed with 0.5 milliequivalent. In fact, the improvement in work output diminished at these concentrations. Statistical tests showed that differences greater than 5 per cent in total work output were significant.

The average differences between the height of contraction of the control and vitamin treated muscles were calculated for each minute of exercise. The average per cent differences⁴ are plotted in figure 2 which shows that as the concentration of calcium pantothenate is increased from 0.001 to 0.5 milliequivalent per liter, the work output is increased significantly during the later stages of exercise. In this chart all differences greater than 15 per cent are statistically significant. Figure 3 is in a similar plot of work distribution for pantothenate concentrations of 0.5, 0.75 and 1.0 milli equivalent per liter. The improvement

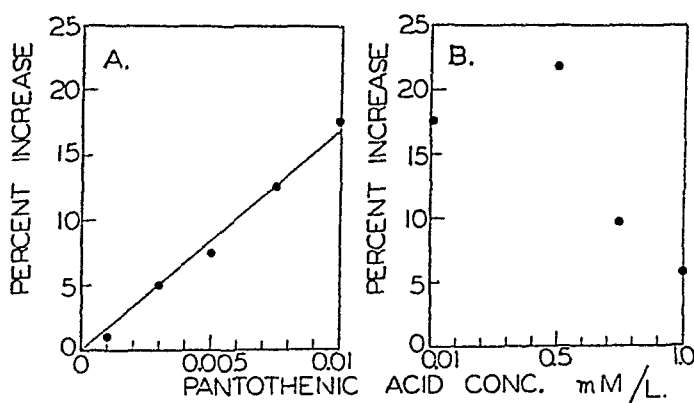


Fig. 1. Effect of changes in concentration of pantothenic acid in the perfusion fluid on the total work output of perfused frog muscles. A. Average per cent increase in work output of muscle perfused with pantothenate over control muscle perfused without pantothenate. Effect of increasing pantothenate concentration from 0.001 to 0.01 milliequivalent per liter. B. Same as A, for pantothenate concentrations up to 1.0 milliequivalent per liter.

in work output diminishes when the pantothenate concentration is increased from 0.5 to 1.0 milliequivalent per liter.

Effect of changes in Ca ion concentration on work output. Although the total calcium content of the control and experimental perfusion fluids were made equal in all experiments, it is possible that the calcium ion content was less in the solutions containing pantothenate than in the control solutions prepared with calcium chloride owing to the incomplete ionization of calcium pantothenate. At concentrations below 0.01 milliequivalent per liter, changes in the calcium ion concentration may be considered physiologically insignificant; since even the frog heart fails to react to changes in calcium ion concentration of less than 0.02 mM per liter (1). The effect of changes in calcium ion concentration on the work output of perfused frog muscles was tested in the following manner. In one set of 10 experiments the Ca concentration was made 1.25 mM per liter

⁴ Calculated as $\frac{\text{Mean difference}}{\text{Mean height of control curve}} \times 100$

(by the addition of CaCl_2) in the perfusion fluid used while the work curve was recorded for the first gastrocnemius muscle and reduced to 0.62 mM per liter while the work curve was recorded for the second muscle. The average total work output for the first legs was 2149 ± 122 gram centimeters while that for the second legs was 1963 ± 82 gram centimeters. The average difference was -186 ± 98 gram centimeters which is not statistically significant. If calcium pantothenate is incompletely ionized, the calcium ion content of the perfusion

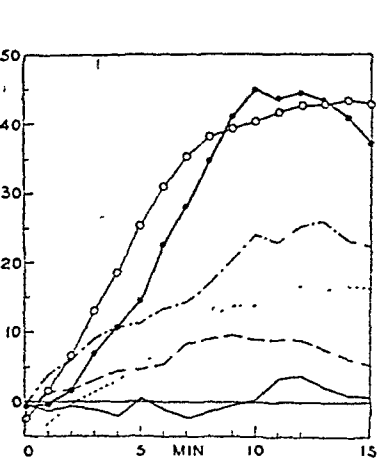


Fig. 2

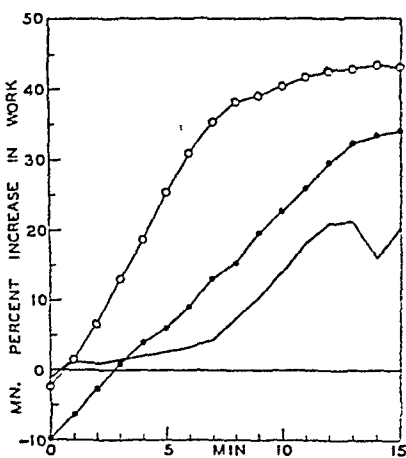


Fig. 3

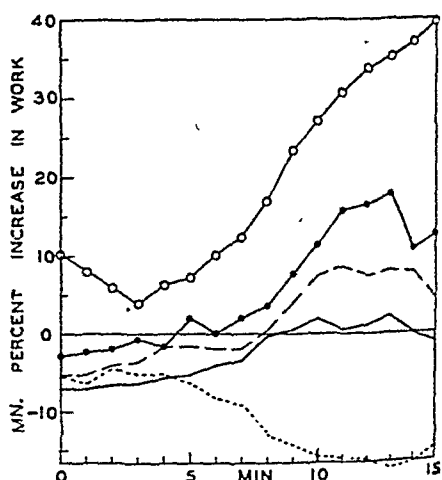


Fig. 4

Fig. 2. Effect of increasing concentration of pantothenate on work distribution of perfused frog muscles. Pantothenate concentrations of: — 0.001 m.eq./l.; - - - 0.003 m.eq./l.; ····· 0.005 m.eq./l.; - · - · - 0.0075 m. eq./l.; ●-●-●-●-● 0.01 m.eq./l.; ○-○-○-○ 0.5 m.eq./l. Each curve represents the average of 10 experiments.

Fig. 3. Effect of increasing concentration of pantothenate on work distribution of perfused frog muscles. Pantothenate concentration of: ○-○-○-○ 0.5 m.eq./l.; ●-●-●-●-● 0.75 m.eq./l.; — 1.0 m.eq./l. Each curve represents the average of 10 experiments.

Fig. 4. Average work distribution in control experiments. ○-○-○-○ 0.5 m.eq./l. of sodium pantothenate added to perfusion fluid of second muscle. ●-●-●-●-● Calcium concentration increased from 0.62 mM per l. for first muscle to 1.25 mM per liter for second muscle. - - - - - Calcium concentration decreased from 1.25 mM per liter for first muscle to 0.62 mM per liter for the second muscle. — — — Control experiments: Both muscles perfused with buffered glucose-Ringer's containing 1.25 mM Ca per liter. — — — Control experiments: Both muscles perfused with buffered glucose-Ringer's containing 1.1 mM Ca per liter.

fluid would be less than calculated for the vitamin treated muscle, and a reduced work output would be expected.

The effect of increasing the calcium ion concentration in the perfusion fluid was also tested in a series of 10 experiments in which the calcium concentration was 0.62 mM per liter for the perfusion of the first leg and 1.25 mM per liter for the second. The average total work output for the first legs was 1893 ± 94 gram centimeters and for the second 1937 ± 87 gram centimeters. The average difference of 44 ± 80 gram centimeters was not statistically significant. In 10

experiments in which the calcium concentration was 1.1 mM per liter in the perfusion fluid used for both muscles the average total work output was 1891 ± 82 gram centimeters for the first and 1822 ± 82 for the second legs. The mean difference of 69 ± 60 was not statistically significant. Similar results were obtained when the calcium concentration of the perfusion fluid was raised to 1.25 mM per liter for both muscles.

Effect of sodium pantothenate on work output. Experiments were also performed in which 0.5 milliequivalent of sodium pantothenate⁵ was added to the perfusion fluid used with the second muscle. In these experiments CaCl_2 was added to the perfusion fluids for both legs to make the total calcium content 1.1 mM per liter. The average total work output obtained in the 10 experiments was 1934 ± 60 gram centimeters in the first leg and 2164 ± 55 gram centimeters in the second. The average difference of 230 ± 60 gram centimeters was statistically significant.

The average work distribution observed in both the sodium pantothenate and the calcium ion concentration experiments is shown graphically in figure 4. In this chart differences greater than 15 per cent are statistically significant. It may be seen that significant improvement in work output follows the inclusion of 0.5 milliequivalent of sodium pantothenate in the perfusion fluids, while alterations in the calcium concentration between 0.62 and 1.25 mM per liter are not accompanied by significant changes in work output.

DISCUSSION. These results indicate a linear relationship between the concentration of pantothenate in the perfusion fluid and the average improvement in work output of perfused frog muscles up to concentrations of 0.01 milliequivalent per liter. Increasing the pantothenate concentration to 0.5 milliequivalent per liter gives only a slightly greater improvement in work output. Further increases in pantothenate concentration (up to 1.0 milliequiv. per l.) diminish the improvement in work output.

From the results of the control experiments it may be concluded that the improvement in work output observed following the addition of low concentrations of calcium pantothenate to the perfusion fluid is not due to changes in the calcium ion concentration.

One possible interpretation of our results is that perfusion of the frog with buffered glucose-Ringer's solution washed out vitamins normally present in the tissues so that the preparation was in a state of acute vitamin deficiency, which was relieved by adding pantothenate (or other vitamins of the B complex) to the second perfusion fluid. We do not believe this to be the case for the following reasons: 1. In control experiments, without the addition of vitamins, the performance of the second leg was not significantly different from that of the first, even though the perfusion had continued for 15 to 25 minutes longer through the second leg than the first. 2. In similar control experiments the perfusion was continued for 60 to 90 minutes after the work curve was run on the first leg before the second leg was exercised. In these experiments no significant differences in work performance were found between the two legs. 3. The addition of

⁵ The sodium pantothenate was supplied through the kindness of Dr. R. T. Major of Merck & Co.

calcium pantothenate to the perfusion fluid in amounts comparable to that found in normal mammalian blood (0.001 mM per liter) did not result in a significant improvement in work output. 4. The work improvement increases with increasing concentration of pantothenate to a concentration of 0.01 milliequivalent. Although direct experimental data are lacking, the evidence available is against the assumption of an acute vitamin deficiency in the muscles used for these experiments.

CONCLUSIONS

1. The addition of calcium pantothenate to the perfusion fluid improved the work output of frog muscles. This improvement increased with increasing concentrations of pantothenate from 0.001 to 0.01 milliequivalent per liter.

2. Further increases in pantothenate concentration of the perfusion fluid to 0.5 milliequivalent per liter resulted in only slightly greater improvement in total work output.

3. Increasing the pantothenate concentration of the perfusion fluid from 0.5 to 1.0 milliequivalent per liter reduced the improvement in work output.

4. Increasing the calcium ion concentration of the perfusion fluid from 0.62 to 1.25 mM per liter did not significantly alter the work output of perfused frog muscles.

5. Decreasing the calcium ion concentration of the perfusion fluid from 1.25 to 0.62 mM per liter was followed by a slight decrease in work output which was not statistically significant.

6. Sodium pantothenate was also effective in increasing work output.

REFERENCES

- (1) McLEAN, F. C. AND A. B. HASTINGS. *J. Biol. Chem.* 107: 337, 1934.
- (2) PETERS, J. P. AND D. D. VAN SLYKE. *Quantitative clinical chemistry*. Vol. II. Methods. Williams & Wilkins, Baltimore, 1932, p. 421.
- (3) SHOCK, N. W. AND W. H. SEBRELL. *This Journal* 142: 265, 1944.

THE EFFECT OF DICUMAROL (3,3'-METHYLENEBIS [4-HYDROXY-COUMARIN]) ON PLATELET ADHESIVENESS¹

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It is believed that platelets play an important rôle in the process of coagulation. They are closely associated with the phenomenon of syneresis and their disintegration releases thromboplastin. Another property that has been noticed by many workers is the adhesiveness of platelets—that is, their tendency to adhere to each other or to surfaces with which they come in contact. Few studies of this property have been made, but H. P. Wright (1) has developed a means of measuring it quantitatively. She suggests that the apparent disappearance of platelets from blood samples standing in glass containers may be due to the platelets sticking to the walls of the vessel.

The method Wright used for the determination of platelet adhesiveness consisted of rotating blood in a glass tube and making platelet counts at various intervals of time. The glass container was so constructed that the sides, which were formed by cover slips held in place with vaseline, could be removed and stained. A vaseline-lined tube was used for control observations. It was found that in the experimental tube the platelet count fell progressively along curves of a logarithmic type, and that the rate of fall depended on the concentration of anticoagulant used. Microscopic examination of the cover slips revealed that they were loaded with platelets. In the control tube, on the other hand, counts decreased only slightly.

The anticoagulants employed in Wright's studies were sodium oxalate, chlorazol dyes and heparin, all of which were used *in vitro*. Our study was undertaken in order to determine whether Dicumarol, an *in-vivo* anticoagulant having no *in-vitro* action, would cause a similar decrease in platelet adhesiveness.

METHODS. The technique used was essentially that described by Wright (1). Four and one-half cubic centimeters of venous blood were drawn into a vaselined syringe and placed in a paraffined beaker containing 0.5 cc. of 2.5 per cent sodium citrate solution. Within ten minutes after the blood was drawn and mixed with the citrate, 1.5 cc. were transferred to a tube similar to the ones used by Wright.²

After the blood was placed in the tube, it was rotated continuously for 80 minutes, except when samples were withdrawn, in a mechanical rotator at approximately 12 r.p.m. Platelet counts, by the direct wet method using Rees-Ecker diluting fluid, were made before the rotation and at 20, 40, 60 and 80

¹ This investigation was aided by a grant from the Wisconsin Alumni Research Foundation.

² Mr. J. B. Davis, glass technician for the departments of chemistry and physics, kindly made this tube for us.

minutes thereafter. It was observed that the counts decreased progressively in a manner similar to that already described. At the end of 80 minutes, the cover slips were removed and stained with Kingsley's stain. Upon microscopic examination they were found to contain large numbers of platelets.

In the present series, each of thirty-three patients was given Dicumarol for a period of six days. Usually four determinations of platelet adhesiveness were made on the patient, one before administration of the drug, two during the administration, and one afterward, when the effect was beginning to decline. The dosage of Dicumarol was gauged by means of prothrombin time determinations. (Pohle and Stewart's modification of the Quick method for prothrombin time was used.) In every instance the prothrombin time was determined on the day before the platelet determinations. By the end of the Dicumarol administration, the prothrombin time in the majority of cases was between 20 and 30 seconds as compared with 10 to 12 seconds in the control.

RESULTS. Table 1 shows a typical study including prothrombin times, amounts of Dicumarol given, and the counts for the determinations of platelet adhesiveness.

Figure 1 is a graph of the average counts of thirty-three patients. It reveals that Dicumarol has no effect on the actual platelet *count*, an observation made previously by Bingham, Meyer and Pohle (2) and by Dale and Jaques (3). The curve representing the adhesiveness determinations before administration of Dicumarol is similar to those obtained by Wright.

The graph shows that adhesiveness definitely decreases after administration of Dicumarol. The data were statistically analyzed by a method in which the differences of the means divided by the standard error of the differences of the means constitutes the criterion of significance. A ratio of two means that the probabilities are five in one hundred that a difference as great as the one observed will be encountered by chance. The differences in the counts of curve A, control curve, and curve C, at the height of the Dicumarol response, are statistically significant at the 20-, 40- and 60-minute intervals. The differences of the means divided by the standard error of the differences of the means are 2.33, 2.03 and 2.38, respectively. At the 80-minute mark the ratio is only 1.66. This figure indicates that the probabilities are ten in one hundred that a difference as great as the one observed will be encountered by chance.

In general the greatest decrease in platelet adhesiveness corresponded to the greatest prolongation of prothrombin time.

DISCUSSION. One hypothesis that has been suggested to explain platelet adhesiveness is that a film of fibrin is formed on the surface of the platelet. If this were true, one would expect the platelets of a hemophiliac to be decidedly less adhesive than normal because of the much slower rate of fibrin formation. In this series a single determination of platelet stickiness was made on hemophilic blood. No difference was observed, however, between this determination and those made on normal blood. This lends support to the idea that the defect in the hemophiliac does not reside in the platelets.

Baronofsky and Quick in a paper entitled "Heparin and the agglutination of platelets in vitro" (4) suggest that Dicumarol may decrease platelet agglutin-

TABLE 1

DATE.....	11-17	11-19	11-20	11-21	11-22	11-23	11-24	11-25	11-26	11-27	11-28	11-29
Prothrombin time.....	10½"				14"/11½"			20½"/11½"			16"/11"	
Platelet determination (Counts in thousands)		7:32 pm. 256 7:52 142 8:12 144 8:32 122 8:52 110				5:22 pm. 248 5:42 126 6:02 110 6:22 110 6:42 110			7:44 pm. 226 8:04 176 8:24 144 8:44 142 9:04 140			6:21 pm. 230 6:41 178 7:01 162 7:21 166 7:41 156
Dicumarol (mgm. per kgm.).....			5	1½	1½	1½	1½	0				

Patients—M. T., male, age 39. Diagnosis—essential hypertension.

ability. In their experiments, in which the prothrombin time of rabbits was prolonged to approximately seven minutes, no tendency toward agglutination was noted. A much larger amount of heparin was needed to prevent agglutination than to prevent coagulation. In the present study, it was observed that in individual cases the prothrombin time was prolonged significantly before the actual decrease in platelet adhesiveness could be detected. There is no direct evidence that platelet agglutination and platelet adhesiveness are identical, yet they appear to be so, or to be closely related.

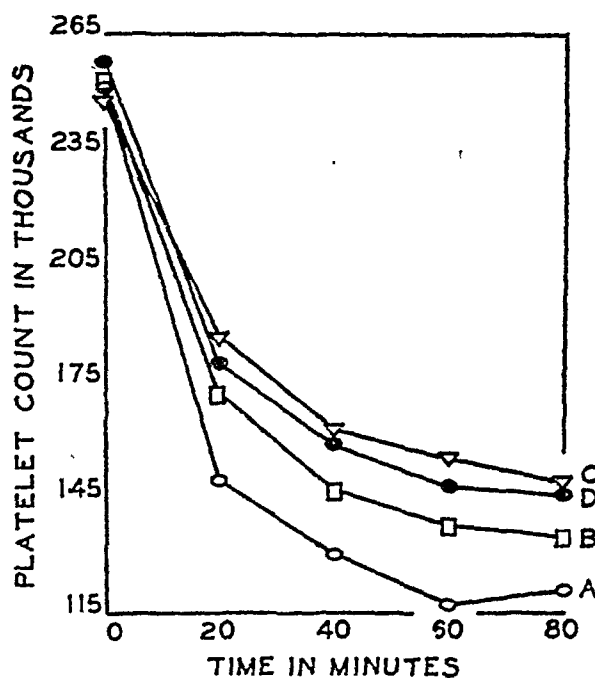


Fig. 1. Platelet adhesiveness as influenced by Dicumarol.

Curve A = Adhesiveness determinations before Dicumarol administration. Prothrombin time 11.4 seconds. Average of thirty-three cases.

Curve B = Fourth day of Dicumarol administration. Prothrombin time 16.6 seconds. Average of thirty-two cases.

Curve C = Day following last dose of Dicumarol. Prothrombin time 22.6 seconds. Average of thirty-three cases.

Curve D = Four days after last dose of Dicumarol. Prothrombin time 18.2 seconds. Average of twenty-eight cases.

Several attempts have been made to determine whether Dicumarol is efficacious as a prophylactic agent of thrombosis. Dale and Jaques (3) produced thrombi in dogs by crushing the saphenous veins, and by inserting a glass cell between the carotid artery and jugular vein. Control dogs all developed thrombi when the vein was crushed, and the glass cell quickly became occluded; Dicumarol-treated dogs had a much lower incidence of venous thrombosis, and the glass cannulae remained patent much longer. Richards and Cortell (5) showed that when lethal doses of Dicumarol were given to dogs, thrombosis did not follow the intravenous injection of Monolate. Thill, Stafford *et al.* (6), in similar experiments, showed that the incidence of experimental thrombosis was mark-

edly reduced by single doses of Dicumarol comparable to those given clinically. Platelet adhesiveness is undoubtedly a factor in thrombosis. The present studies offer additional evidence in favor of the use of Dicumarol prophylactically, since a definite decrease in platelet adhesiveness has been observed in addition to the hypoprothrombinemia, which is probably important in itself.

In studies of the changes in platelet adhesiveness that follow parturition and surgical operation, Wright (7) observed an increased stickiness at the time when, statistically, thrombosis is likely to occur. There was also a simultaneous increase in platelet count. On the basis of these findings she postulated that the large numbers of newly formed platelets which were released into the blood stream were hyperadhesive. Whether Dicumarol prevents the increase in adhesiveness after surgical operation remains to be determined.

SUMMARY

1. Dicumarol has no effect on the platelet count per se.
2. Dicumarol definitely decreases the adhesiveness of platelets as measured by the method of Wright.
3. Evidence in favor of Dicumarol as a prophylactic agent of thrombosis is discussed.

REFERENCES

- (1) WRIGHT, H. P. *J. Path. and Bact.* 53: 255, 1941.
- (2) BINGHAM, J. B., O. O. MEYER, AND F. J. POHLE. *Am. J. Med. Sc.* 202: 563, 1941.
- (3) DALE, D. U. AND L. B. JAQUES. *Canad. M. A. J.* 46: 546, 1942.
- (4) BARONOFSKY, I. D. AND A. J. QUICK. *Proc. Soc. Exper. Biol. and Med.* 53: 173, 1943.
- (5) RICHARDS, R. K. AND R. CORTELL. *Proc. Soc. Exper. Biol. and Med.* 50: 237, 1942.
- (6) THILL, C. J., W. T. STAFFORD, M. SPOONER AND O. O. MEYER. *Proc. Soc. Exper. Biol. and Med.* 54: 333, 1944.
- (7) WRIGHT, H. P. *J. Path. and Bact.* 54: 461, 1942.

THE OXYGEN CONSUMPTION OF NORMAL RAT LIVER AND DIAPHRAGM MUSCLE IN LYMPH TAKEN FROM DOGS BEFORE AND AFTER SEVERE BURNS

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In a previous paper Muus and Hardenbergh (1) showed that lymph from a severely burned leg caused an increase in the oxygen consumption of rat liver slices as compared with slices of the same liver in lymph collected from the leg before it was burned. Most of the observations were made on lymph from calves burned by immersion of the leg in boiling water.

These first experiments were part of a search for substances in the exudate from burned regions which might show biological effects of some sort. Burning by immersion in hot water does not break the skin, and provides an experimental situation in which infection and loss of exudate from the skin surface do not enter. The lymph is thus the uncomplicated exudate from the lesion, and should contain any abnormal substances formed in the burn in maximum concentration. Even though there is apparently no interference with the normal relation of the part with the circulation (2), such substances should be present in the lymph collected from vessels just beyond the limits of the burn in a higher concentration than in the blood from the general circulation. The results seemed to justify this assumption.

In the course of many experiments upon calves and dogs anesthetized with nembutal and burned severely, we have been impressed by the fact that after the burn the body temperature may begin to rise. Unless the rise in temperature is checked, which was done by keeping the animal wet with water and in the draught from an electric fan, this tendency may result in hyperpyrexia and death. The same tendency toward a rise in body temperature has been noted in human patients after burns and has resulted in the more cautious use of heat in the shock-like condition which so often accompanies extensive burns.

With this in mind we decided to investigate the oxygen consumption of voluntary muscle in lymph from burned animals by a procedure similar to that used with liver slices. For these experiments we have used lymph collected from the hind legs of dogs before and after burning. The experiments also served to extend the observations on liver slices from calf lymph to dog lymph. One such experiment has been briefly mentioned (1), but the technique whereby it was possible to obtain a sufficient amount of lymph from dogs before as well as after burning has not been described, and an account may prove useful to other workers.

EXPERIMENTAL. (a) *Collection of lymph.* Cannulation of lymphatics in the legs of anesthetized dogs just above the ankle joint, and collection of lymph

before and after the feet have been burned by immersion in hot water have been employed frequently in this laboratory. This method might provide enough lymph for the experiments on oxygen consumption after the burn, when increased capillary leakage provides a free flow of lymph which can be further increased by mild passive motion. But one experiences the greatest difficulty in collecting 10 to 15 cc. of normal lymph for control from so small an area, particularly as the use of an anticoagulant while collecting is undesirable. To overcome this difficulty we have proceeded as follows: Dogs weighing 11 kgm. and upwards are anesthetized and kept under complete anesthesia throughout the experiment by intravenous injection of 5 per cent nembutal, 35 mgm. per kilo of animal. The abdomen is opened by a mid-line incision, which should reach from the tip of the sternum to about 5 cm. above the symphysis. The animal is then placed in a head-down position at an angle of 45° . If the incision is held wide open and the intestines are reasonably empty, the abdominal contents slide gently toward the diaphragm and remain out of the operating field without necessitating use of more than a slight amount of gauze packing.

In the dog the receptaculum chyli lies behind and between the aorta and inferior vena cava from the level of the renal vessels to a point several centimeters below the lower margins of the kidneys. No precise level can be given, since the receptaculum simply decreases in diameter to become one or sometimes two large trunks. These two, or more often a single one, lie just as does the receptaculum, covered by the aorta and inferior cava and more or less tightly adherent to one or the other of them, usually the aorta. There are no valves in the receptaculum, and none in the large vessel leading into it which is to be cannulated. In our experience, the extreme delicacy of the wall of the receptaculum and its adherence to the blood vessels make it very difficult to get a ligature around it, and if it is wounded the operator will be confronted by a gush of chyle which is usually uncontrollable and always enormously annoying. Furthermore, if the lymph from the small intestines runs freely into the peritoneal cavity during the experiment, it will mean a steady loss of plasma from the circulation, and this is not to be permitted in the face of a serious burn from which it is desired to collect lymph over 5 to 9 hours. The operator must isolate the lymphatic or the lymphatics below the receptaculum and tie them. Since these vessels, though valved ineffectually, have thicker walls than the receptaculum, they can be dissected from the aorta, and as they communicate freely cannulation of the larger will produce the lymph from both. The dissection to isolate and cannulate this vessel is time-consuming because of many small blood vessels in the region, the delicacy of the wall of the lymphatic, and the close attachment to the aorta or vena cava. Indeed, part of the lymphatic wall after it is freed and encircled by a ligature is usually connective tissue split off the adjacent blood vessel. An L-shaped cannula is employed, with an opening of 1 to 2 mm. in diameter in the short leg of the L. The long leg extends out through the abdominal incision, to the left side of which it is fastened by a stitch so as to hold it in correct position. The animal is then lowered into a horizontal position and the abdominal contents gently disposed in their normal place and with the

cannula in their midst. The hind feet are then attached to a bar which rotates in a circle with a diameter of 40 cm. twelve times a minute. This results in rhythmic passive extension and flexion of the legs upward and at right angles to the body when extension is complete. This passive movement causes continuous flow of lymph before burning and greatly accelerates lymph flow after burning. The lymph collected amounts to practically all that from the hind legs and tail, plus very small increments from the lower part of the large intestine, the genitalia, and the bladder. It is unfortunate to be compelled to enter the abdomen but unavoidable since no cannulation along the femoral vessels gives more than a fraction of the total lymph from one leg, and the single cannulation in the abdomen collects all the lymph from both legs.

(b) *Technique of burning.* It is obvious that the position of the cannula, fastened upright in the center of the abdomen with the tip at right angles to the shaft and tied into an extremely fragile vessel, is precarious at best, and becomes more so if the animal is moved to any degree in order to accomplish the extensive immersion in hot water required by the experiment. In order to burn for a precise period of time and maintain lymph collection unbrokenly, it has been found best to use a rubberized fabric sleeve which is 19 cm. in diameter at one end and about three times that at the other. The smaller end is slipped over the dog's hind legs and tail to a level just below the end of the abdominal incision. It is fastened against the animal by a wide ribbon of rubber sheeting tied around the body of the dog and overlapping the upper edge of the sleeve. The lower, wide end is looped upright to form the mouth of a funnel into which hot water may be poured *en masse*, and by suddenly lowering the funnel end the water may be dumped out with equal abruptness. This results in burning both hind legs, the skin over the rear of the dog, the lower part of the abdomen, and the external genitalia for a precise time and without moving the dog or endangering the cannulation.

(c) *Oxygen consumption.* The oxygen consumption was measured by the direct Warburg technique. The lymph was first allowed to clot and the fibrin removed. If fibrin formation was not complete before the lymph was introduced into the vessel, a film of fibrin would form on the surface of the tissue slices and completely invalidate the results. A complete clot always formed very shortly in the normal lymph, but the lymph collected after burning seldom clotted spontaneously. Calf lymph had often shown increased clotting time after burning and gentle stirring was necessary to promote clotting. Though the time of burning used on the dogs was shorter, the effects of the burn were more severe by all accounts, and the impairment of the clotting mechanism was definitely more pronounced. In most cases complete clotting could only be secured by adding tissue extract. A crude saline extract of beef lung was added in an amount of from 0.1 to 0.2 cc. per 10 cc. of lymph. When this was done a similar amount was added to the normal lymph. A pure thrombin preparation was used in a few cases but was found less effective.

The lymph serum was freed from carbonate by addition of acid and evacuation according to Friend and Hastings (3) and adjusted to pH 7.4.

A 2 cc. sample of lymph was used in each vessel, and 10 to 15 mgm. dry weight of liver slices or 20 to 30 mgm. dry weight of diaphragm. All tissues were taken from normal, well fed rats. Control vessels with lymph and no tissue were run simultaneously. Potassium hydroxide, 0.2 cc. 20 per cent, was placed in the center cup, and 0.1 cc. 4 per cent glucose was added to the medium. The experiments were done at 37°C., and the oxygen consumption measured for 60 minutes. Q_{O_2} was computed as cmm. O_2 per hour per milligram dry weight of tissue at the end of the experiment. The rate of oxygen consumption of the liver slices remained constant during this period. In some cases the oxygen consumption for diaphragm fell slightly during the later part of the experiment, and the Q_{O_2} reported is computed for the period during which the rate remained constant, which was never less than 40 minutes.

(d) *Effect of hemolysis.* The lymph collected from dogs after burning was always strongly colored with hemoglobin. This complication had not been present when calf lymph was used. In a few cases only had the lymph been slightly colored during the first $\frac{1}{2}$ to 1 hour, and such samples had never been used in the metabolism studies. As the effect on respiration manifested itself much earlier in the experiments on dogs, the possibility that it was caused by the products of hemolysis with which the lymph was so grossly contaminated had to be considered. A few experiments were, therefore, carried out on the effect of hemolysis on the oxygen consumption of liver slices. Heparinized blood from a normal dog was centrifuged and the cells separated. The cells were hemolyzed by being frozen and thawed, and were diluted with serum from the same dog. The mixture was centrifuged to remove unhemolyzed cells, and appropriate dilutions of the clear solution and serum were made. The samples used in the Warburg apparatus were treated as just described for lymph. Hemoglobin was determined by the method of Evelyn (4).

RESULTS. The results are summarized in tables 1 and 2. Each set of results was obtained simultaneously on samples from the same liver or pieces of the same diaphragm. All determinations were done in duplicate. With two determinations and one blank for each lymph sample, the maximum number of samples that could be used with a 14-vessel Warburg apparatus was four. The blank in this series of experiments never showed any oxygen consumption, and in the first group of experiments on dog 4 it was therefore deemed safe to do five duplicate determinations without including the blank, and check the blank on the following day when the determination on the diaphragm required only four vessels.

Two values for Q_{O_2} were determined for diaphragm in each set, one with normal lymph and one with the lymph sample in which liver showed the highest Q_{O_2} . The number of determinations was limited to two, because no more than four pieces large enough to give a reasonable oxygen consumption could be secured from one diaphragm. The total number of experiments on lymph from one dog was limited by the amount of normal lymph, which was usually about 12 to 14 cc. The collection of normal lymph had to be discontinued when there was obvious danger of clotting in the cannula. After the animal was burned this danger was removed because of the decreased tendency to clot.

TABLE 1

Oxygen consumption of rat liver and diaphragm in lymph from the hind legs of burned dogs

NUMBER OF DOG	TIME OF BURN	DURATION AFTER BURN	TIME LYMPH TAKEN AFTER BURN	HEMOGLOBIN	QO ₂	
					Liver	Diaphragm
	<i>min.</i>	<i>hours</i>	<i>hours</i>	<i>gm. per 100 cc.</i>	<i>cmm. per mgm. per hour</i>	
1	1	6	Normal lymph 3 to 5	0.0	9.8	4.6
				0.7	10.9	4.7
2	3 $\frac{1}{4}$	5 (died)	Normal lymph 2 to 3	0.0	9.4	4.6
				1.1	13.0	5.6
3	$\frac{3}{4}$	3 (died)	Normal lymph 1 to 2 2 to 3	0.0	12.9	4.2
				0.3	14.5	5.1
				0.4	13.9	
4	$\frac{1}{2}$	9	Normal lymph 2 to 2 $\frac{1}{2}$ 4 to 5 5 $\frac{1}{2}$ to 6 $\frac{1}{2}$ 7 to 8	0.0	9.8	
				0.7	10.3*	
				0.6	10.3	
				0.6	11.5	
				0.5	10.9	
			Normal lymph $\frac{1}{2}$ to 1 2 to 2 $\frac{1}{2}$ 5 $\frac{1}{2}$ to 6 $\frac{1}{2}$	0.0	9.5	4.7
				0.2	10.7	
				0.7	10.6*	
5	1	8	Normal lymph 0 to 1 3 $\frac{1}{2}$ to 6 6 to 8	0.0	8.4	4.6*
				0.5	10.3	
				0.9	11.7	6.5
				0.9	11.3	

* Single determination.

TABLE 2

Effect of hemolysis on the oxygen consumption of rat liver slices

MATERIAL	HEMOGLOBIN	QO ₂
	<i>gm. per 100 cc.</i>	<i>cmm. per mgm. per hour</i>
Normal serum.....	0.0	10.9
Normal serum + hemolyzed cells	1.6	10.9
Normal lymph.....	0.0	11.1
Normal lymph + hemolyzed cells	1.1	10.8
Normal serum.....	0.0	10.8
Normal serum + hemolyzed cells	0.8	11.3
Normal serum + hemolyzed cells.....	1.5	10.8

The results from the experiments with liver differ from those previously reported with calf lymph in that the effect on QO₂ can be demonstrated much earlier and seems to reach a maximum in 3 to 6 hours. With calf lymph very little

effect could be demonstrated after 4 hours, and the effect increased at least up to 22 hours. As already mentioned, the dogs showed a pronounced effect of the burn which though shorter was much more extensive, and the severe hemolysis may be taken as a sign of extensive tissue damage.

Table 2 shows that hemolysis *per se* has no effect on the Q_{O_2} of liver slices. The range of hemoglobin concentrations used is even somewhat greater than that found in the burned lymph.

The number of determinations on diaphragm is not large, but the results show that burned lymph which is effective in increasing the liver metabolism has a similar effect on muscle metabolism. In four out of five experiments there is a definite increase in the Q_{O_2} for diaphragm, and in the one experiment where there is no measurable difference, the increase with liver is only very slight.

SUMMARY

A technique whereby it is possible to collect 10 to 15 cc. of normal lymph from the hind legs of dogs without the use of an anticoagulant is described.

The lymph collected from this area after it had been severely burned was shown to increase the oxygen consumption of rat liver slices as compared with slices from the same liver in normal lymph. Such an effect had previously been demonstrated for calf lymph.

The lymph from the burned legs was found to cause a similar increase in the oxygen consumption of rat diaphragm muscle.

REFERENCES

- (1) MUUS, J. AND E. HARDENBERGH. J. Biol. Chem. **152**: 1, 1944.
- (2) GLENN, W. W. L., H. H. GILBERT AND C. K. DRINKER. J. Clin. Investigation **22**: 609, 1943.
- (3) FRIEND, D. G. AND A. B. HASTINGS. Proc. Soc. Exper. Biol. and Med. **45**: 137, 1940.
- (4) EVELYN, K. A. J. Biol. Chem. **115**: 63, 1936.

CHANGES IN PHOSPHATE OF MUSCLE DURING TOURNIQUET SHOCK¹

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Many reports on shock indicate that the absorption of products of tissue autolysis from the area of injury may be an important factor in the development of shock (1). While no toxic substance has been isolated and definitely identified from the blood of man or animals in shock, there are many experiments which indicate the possibility of the production of shock by blood carrying toxic substances from regions of tissue autolysis (2). Green (3) made an extensive study of shock produced after the release of tourniquets which occluded the flow of blood to the hind limbs of animals for several hours. He found that shock was not produced unless occlusion was maintained for more than three hours before release and that intravenous administration of plasma did not prevent shock nor did repeated administration of plasma prevent death. Shock was prevented by amputation or tight binding of the limbs shortly after the release of the tourniquets. Animals recovered from shorter periods of application of tourniquets and many short periods with recovery periods could be instituted without lethal effect. Green prepared extracts of normal muscle which were toxic on intravenous injection and produced a shock syndrome that terminated fatally on intramuscular injection. Fresh muscle was immediately precipitated with acetone and the dried residue extracted with saline solution contained the toxic substance, which could be further purified by additional precipitation procedures. The extract from muscle equivalent to a fifth of the body weight produced shock when injected intramuscularly into rats.

Further purification of the muscle extracts suggested that the toxic substance was adenosine triphosphate and Bielschowsky and Green (4) isolated and identified this substance. The physiologic properties of pure adenosine triphosphate and of that isolated from normal muscle were similar. The amount of adenosine triphosphate isolated from normal muscle diminished rapidly with time after removal of the fresh muscle. Very little toxic material or adenosine triphosphate could be extracted from muscle that had been occluded with a tourniquet for three hours or longer.

Buell and her associates (5) found a very rapid hydrolysis of phosphocreatine and adenosine triphosphate in autolyzing muscle. In their preparations which were powdered while frozen, only a trace of these substances remained after fifteen minutes in saline solution at room temperature. In intact muscle contracting three times each second we (6, 7, 8) found a rapid accumulation of

¹ Read before the Division of Biological Chemistry of the American Chemical Society at Cleveland, Ohio, April 3-6, 1944.

inorganic phosphate and a decrease of phosphocreatine and adenosine triphosphate. The maximal changes occurred in the first two minutes of work when more than 80 per cent of the phosphocreatine and 60 per cent or more of the adenosine triphosphate were hydrolyzed. Continuation of work for several hours did not bring any further changes but when rest ensued there was a rapid decrease of the inorganic phosphate of the muscle and phosphocreatine was reconstituted within a few minutes. Regeneration of adenosine triphosphate was less rapid than regeneration of phosphocreatine but was almost complete within one hour. The products of hydrolysis of phosphocreatine or adenosine triphosphate did not appear to be washed out of the muscle into the blood during the periods of work or rest.

The relation of the phosphates of muscle to shock produced by the release of a tourniquet from occluded muscle poses several questions. What changes occur in the phosphate compounds of muscle during the time the blood flow to the muscle is restricted? How long are these changes reversible as in exercising muscle and at what time do they become irreversible? What substances are washed out of the previously occluded muscle by the blood? Does the occurrence of shock in animals after release of tourniquets from muscle correlate with the data suggested in the foregoing questions? The data presented in this paper deal with these questions.

EXPERIMENTAL PROCEDURE. Male rats weighing from 200 to 250 grams were anesthetized with pentobarbital sodium. A rubber band was wrapped tightly about the thigh and moved to the upper part of the thigh with the aid of a hemostat acting as a lever. In all cases there was sufficient pressure to overcome the arterial pressure. In all animals used there was no appreciable swelling of the limb during the period of constriction such as occurs when the constriction is less than the arterial pressure. If the animals were to survive for many hours after release of the constriction only one leg was occluded but in the experiments involving shock one hind leg and both forelegs were occluded and the muscle from the unoccluded leg was used to determine any effect of shock on the muscle. All specimens of muscle were obtained by freeing the skin from the leg a few minutes prior to obtaining the specimen. At the desired time the Achilles tendon was severed and pulled upward, and the flexor muscles of the leg were severed with one stroke and dropped immediately into a mixture of carbon dioxide snow and alcohol. The frozen muscle was powdered between cooled steel blocks and transferred frozen to tared tubes containing ice-cold 5 per cent solution of trichloroacetic acid and glass beads. The tubes were shaken in a room at 0°C. for fifteen minutes and the solution was filtered. The acid-soluble phosphates were fractionated with barium hydroxide at 0°C. according to a modified Eggleton and Eggleton (9) technic; the barium-insoluble phosphates were dissolved with trichloroacetic acid and reprecipitated with barium hydroxide. The phosphate content was determined by the method of Fiske and Subbarow (10).

RESULTS. The changes of the total acid-soluble phosphorus, inorganic phosphorus, the acid-labile portion of adenosine triphosphate and phosphocreatine

in muscle after periods of complete occlusion of the blood supply are given in table 1 and figure 1. There was no appreciable change of the total acid-soluble phosphorus. The inorganic phosphate rapidly increased and reached a maximal

TABLE 1
Phosphates of muscle after application of tourniquet

TIME OCCLUDED	RATS	HEMA-TOCRIT	PLASMA INORGANIC PHOSPHATE	MUSCLE				
				Total acid-soluble P	Inorganic P	Acid-labile P	Phospho-creatine-P	Water
minutes		per cent	mgm. per 100 cc.	mgm. per 100 grams	mgm. per 100 grams	mgm. per 100 grams	mgm. per 100 grams	per cent
0	30			146 ±1.4*	31.7 ±0.8	40.3 ±0.9	50.3 ±1.0	
15	10	39	6.38 ±0.13	154 ±3.0	61.9 ±2.1	35.7 ±1.3	26.1 ±1.6	76.0 ±0.7
30	2	39	6.26	156	65.1	34.7	20.8	77
60	8	36	6.93 ±0.21	149 ±4.2	73.9 ±1.3	31.5 ±1.6	4.5 ±0.8	75.9 ±1.7
180	7	34	7.05 ±0.52	148 ±3.3	99.9 ±2.6	5.22 ±1.0	3.6 ±0.8	76.4 ±0.5
240	8	37	8.81	147 ±2.8	113.5 ±2.5	3.8 ±0.8	2.4 ±0.1	77 ±0.5

* The value following the ± sign is the standard error of the mean.

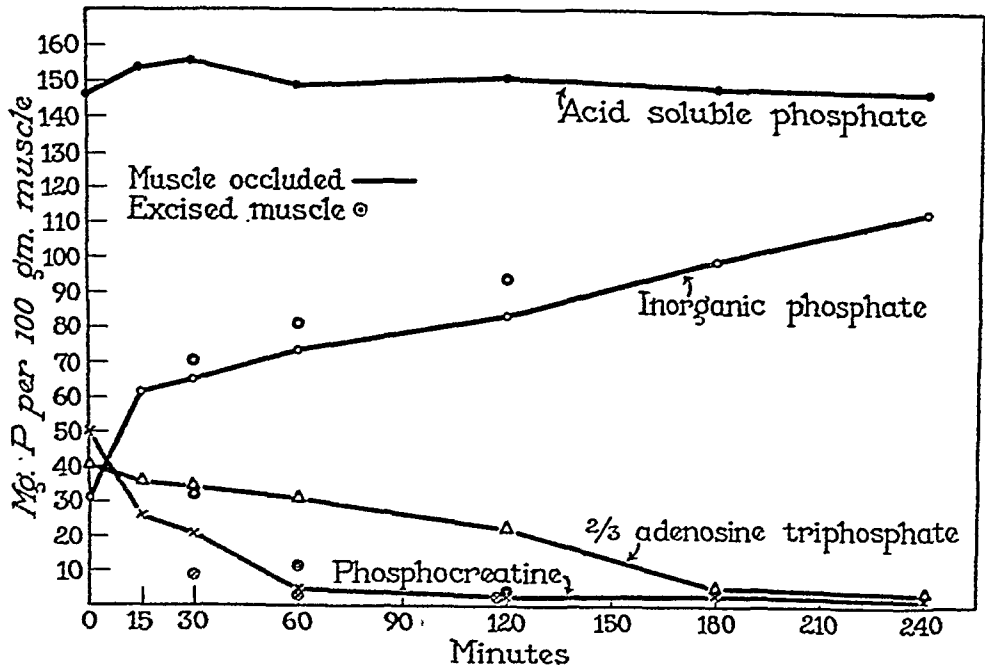


Fig. 1. Changes in phosphate compounds of muscle after occlusion of the blood supply. Average figures obtained from analysis of leg and thigh muscles of adult rats at the times indicated after complete occlusion of the blood supply by a tourniquet at 0. The circled figures denote the changes in similar muscles excised at 0 and maintained at body temperature until the times indicated.

figure after four hours. Adenosine triphosphate gradually hydrolyzed almost to completion in about three hours, while the hydrolysis of phosphocreatine was nearly completed in about one hour. There was little change of the water content of the occluded muscle.

The changes that occurred in previously occluded muscle after the reestab-

lishment of the circulation to the muscles are given in table 2 and figure 2. The return of blood flow after sixty minutes or more of occlusion produced edema

TABLE 2
Phosphates of muscle after release of tourniquet

TIME OCCLUDED	TIME RELEASED	RATS	HEMATOCRIT	PLASMA INORGANIC PHOSPHATE	MUSCLE				
					Total acid-soluble P	Inorganic P	Acid-labile P	Phospho-creatine-P	Water
minutes	minutes		per cent	mgm. per 100 cc.	mgm. per 100 grams	mgm. per 100 grams	mgm. per 100 grams	mgm. per 100 grams	per cent
15	15	6	37	6.26	149 \pm 5.4*	44.7 \pm 3.8	35.0 \pm 0.9	38.8 \pm 2.4	76.6 \pm 0.6
60	60	8	39	7.65 \pm 0.18	130 \pm 7.5	28.3 \pm 1.0	29.7 \pm 1.1	39.3 \pm 2.4	78.9 \pm 0.8
180	60	7	56	15.56 \pm 1.49	102 \pm 5.7	58.8 \pm 9.6	13.4 \pm 3.6	14.2 \pm 3.6	82.2 \pm 0.4
240	15	2	40	11.08	89.6	66.2	5.6	4.9	84.0
240	60	4	53	12.54 \pm 1.05	93.6 \pm 9.8	67.6 \pm 7.2	4.3 \pm 1.2	6.7 \pm 1.7	82.7 \pm 9.2
240	120	13	60	19.00 \pm 1.66	74.7	55.9 \pm 5.7	5.5	3.9	82.8 \pm 0.7
240	360	4	60		53.4	46.1 \pm 10.0	5.9 \pm 1.1	3.7 \pm 0.7	83.4 \pm 0.5
	hr.								
240	22†	4	34	10.36 \pm 2.05	34.4 \pm 9.5	20.2 \pm 6.7	4.8 \pm 1.5	3.2 \pm 1.5	83.9 \pm 0.7
240	46†	4	38	8.08 \pm 0.48	31.2 \pm 2.3	19.1 \pm 1.6	3.3 \pm 0.3	2.6 \pm 0.4	85.0 \pm 0.9
240	120†	4	26	6.44 \pm 0.49	56.5 \pm 8.0	32.0 \pm 8.1	6.8 \pm 1.9	2.1 \pm 1.3	83.1 \pm 1.0
240	168†	6	39	3.72 \pm 0.13	62.0 \pm 4.0	18.3 \pm 0.9	12.3 \pm 0.9	10.3 \pm 2.1	83.3 \pm 0.6
240	264†	2	33	3.52	67.3	26.8	9.5	10.7	81.0

* The value after the \pm is the standard error of the mean.

† Two forelegs and one hind leg were occluded in all experiments except those marked † when only one hind leg was occluded.

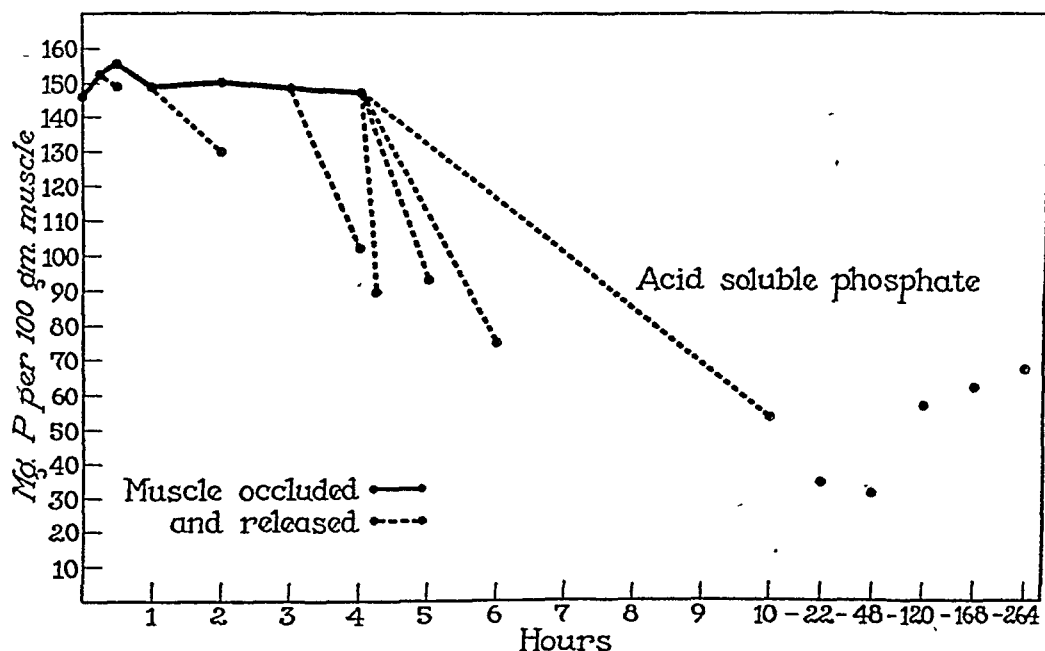


Fig. 2. Decrease of acid soluble phosphate content of muscle after release of occlusion of the blood supply. The first point of each dotted line indicates the time of release of the occlusion and the second point indicates the time the muscle was removed for analysis. The decrease observed on release of occlusion for one hour represents dilution due to increased water content of the muscles. After three hours' occlusion and release there is dilution with water and also considerable washing out of inorganic phosphates.

of the muscle which was obvious within a few minutes and the water content of the muscle was increased. There was a corresponding dilution of the acid-

soluble phosphates of the muscle. If the muscle had been occluded for not more than three hours there was an appreciable resynthesis of adenosine triphosphate and phosphocreatine. This resynthesis was more complete after periods of occlusion shorter than three hours. When the occlusion had persisted for more than three hours there was no resynthesis of these compounds.

After occlusion of three or four hours' duration there was a rapid washing out of the inorganic phosphates from the muscle in addition to the dilution which occurred with the development of edema. The inorganic phosphates of the plasma were increased and there was hemoconcentration. There was no indication that any adenosine triphosphate or phosphocreatine was washed out of the muscle by the blood. It should be noted here that these figures were obtained from animals which had many signs of shock but which had survived without collapse for the period indicated. In these animals the amount of

TABLE 3

Phosphates of unoccluded muscle after release of tourniquet from other legs

OTHER LEGS		RATS	HEMA- TOCRIT	PLASMA INORGANIC PHOSPHATE	MUSCLE				
Time oc- cluded	Time re- leased				Total acid- soluble P	Inorganic P	Acid-labile P	Phospho- creatine P	Water
minutes	minutes		per cent	mgm. per 100 cc.	mgm. per 100 grams	mgm. per 100 grams	mgm. per 100 grams	mgm. per 100 grams	per cent
15	0	4	38	6.27 \pm 0.21*	144 \pm 2.3	37.5 \pm 2.6	35.4 \pm 0.6	44.6 \pm 1.9	76.5 \pm 0.6
180	60	7	56	15.56 \pm 1.49	159 \pm 1.6	42.4 \pm 1.7	37.4 \pm 1.6	41.0 \pm 2.1	76.0 \pm 0.6
240	15	2	40	11.08		31.7	30.8	34.7	
240	60	4	53	12.54 \pm 1.05	156 \pm 3.9	37.6 \pm 3.6	36.9 \pm 2.2	42.2 \pm 1.5	75.0
240	120	13	60	19.00 \pm 1.66	153	40.8 \pm 2.6	40.7	37.0	76.5 \pm 0.6
240	360	4	60		165 \pm 2.4	58.4 \pm 6.7	38.4 \pm 0.2	31.0 \pm 4.7	75.0 \pm 0.9

* The value following the \pm sign is the standard error of the mean.

inorganic phosphate washed out of the muscle was definitely greater than in the rats that showed early collapse and survived shorter periods of time after the release of the occlusions.

The phosphates of muscle obtained from the unoccluded hind leg at various periods after the release of the occlusions from the other three legs are given in table 3. There appeared to be a definite increase of the inorganic phosphate of this muscle and a moderate decrease of its phosphocreatine content. From the individual data of this series it would appear that these changes depend on the low blood pressure rather than on a simple transference of inorganic phosphate from the previously occluded muscle to the normal resting muscle.

COMMENT. The changes of the phosphate compounds of muscle that occur after complete occlusion of the blood supply are similar to those which have been shown to occur in autolyzing muscle. Such changes appear to be almost complete within a few minutes in ground muscle incubated in saline solution but require about two hours in excised muscle, the structure of which has not been greatly disturbed. In a small series of rats killed by ligation of the base of the heart, we found that about three hours were necessary for these autolytic

processes to reach completion. In muscle after complete occlusion of the blood supply there is no change of the total acid-soluble phosphates but there is a conversion of organic phosphates to inorganic phosphate. Most of this phosphorus is accounted for by the hydrolysis of phosphocreatine and adenosine triphosphate. The phosphocreatine decreases gradually from the time of occlusion and almost disappears within one hour. Adenosine triphosphate disappears in about three hours.

If the blood flow is returned to the muscle after less than three hours of occlusion there is a reversal of these chemical changes with reconversion of the inorganic phosphate to phosphocreatine and adenosine triphosphate. The hydrolysis and resynthesis of these compounds with occlusion and release are similar to those which occur with muscular exercise and rest except that the hydrolysis may be accomplished much more rapidly by work than by occlusion. There is also functional recovery of muscle occluded less than three hours but this may be difficult to demonstrate until the edema of the muscle has subsided, which takes three or four days. After a week there seems to be complete recovery of the muscle and comparisons of the muscles of the occluded leg with the normal of the same animal do not show any differences.

If the blood flow is returned to muscle occluded for four hours there is no regeneration of phosphocreatine or adenosine triphosphate and a large amount of inorganic phosphate is washed out of the muscle. If fatal shock has not been produced it is several days before there is an appreciable increase of the acid-soluble phosphates of the muscle. From studies of microscopic sections of muscle taken several days after release from occlusion, it is apparent that muscle fibers are beginning to regain their normal staining reactions. After two weeks or more there is a return of the functional capacity of the injured leg. In four to six weeks the rat appears to use this leg as well as a normal member but in all cases we have observed even after three months the muscles have not regained their normal size.

In these studies we did not observe any rats dying after several days from renal impairment as described by Bywaters (11) in cases of crushing injury. This may suggest that the human kidney is much more susceptible to injury from myohemoglobin than is the kidney of the rat or that the two conditions are not entirely comparable. Our animals which were not killed for chemical studies died with shock in less than twenty-four hours or, if only one leg was occluded, they survived with complete or partial recovery of function of the leg. Our experience with adult white rats is similar to that of others as to the survival after release of complete occlusion under various circumstances. These may be summarized as follows: In rats anesthetized with pentobarbital sodium and maintained at room temperature, when the occlusion of one leg and thigh is released after occlusion for four hours most animals show some signs of shock and hemoconcentration but usually recover. Release after six hours of occlusion is usually fatal within twelve hours after release.

After release of occlusion of both hind legs or one hind leg and both forelegs recovery occurs if the occlusion was maintained for less than three hours. After

occlusion of three hours' duration some animals die within a few hours but others recover. After occlusion of three and a half or more hours all animals have died within ten hours after release of the obstruction, usually in about three hours. Intravenous injections of rat plasma will prolong the survival time of these animals but we have been unable to extend their survival for more than twenty hours. Occlusions for two hour periods with release for thirty minutes were repeated four times with subsequent survival and recovery. There are considerable imbibition of fluid in the muscles after release from occlusion of two hours' duration and considerable regeneration of the organic phosphate compounds of the muscle. We were unable to demonstrate that preliminary short periods of occlusion and release produced any increased resistance of the animals to shock after release from occlusions of four hours' duration.

Stimulation of muscle after occlusion of its blood supply will produce about 200 contractions. Chemical changes may be produced in one minute by stimulation after occlusion similar to those which require about three hours when the blood supply is occluded without stimulation. Resynthesis does not occur unless the constriction is released. We stimulated electrically both hind legs of twenty-seven rats immediately after occlusion and released the occlusion after two hours. Sixteen died with shock in from two to eighteen hours after the release of the constriction and eleven recovered. None of our rats have died after release from similar occlusion of two hours without stimulation. Mechanical trauma to the muscle immediately after occlusion also shortens the time occlusion is necessary to produce fatal shock after release of the occlusion. We have been unable to demonstrate any significant decrease of the time of occlusion necessary to produce fatal shock after release of the occlusion when only one leg has been occluded and stimulated. If a toxic substance develops from the hydrolytic products produced by exercise, this substance is not formed in great amounts or is also destroyed during the autolytic processes.

SUMMARY

The changes which occur in the phosphates of muscle the blood supply of which has been completely occluded are those of autolyzing muscle. Adenosine triphosphate almost disappears after three hours and phosphocreatine is almost completely hydrolyzed in one hour. The inorganic phosphates of the muscle rapidly increase to a maximum in about four hours. The total of the acid-soluble phosphates is not changed. If the flow of blood is restored to the muscle within three hours there is resynthesis of adenosine triphosphate and phosphocreatine with a corresponding decrease of the inorganic phosphate. Fatal shock does not develop even though large amounts of muscle have been occluded. When the occlusion is released after more than three hours there is no regeneration of adenosine triphosphate or phosphocreatine but considerable inorganic phosphate is washed from the injured muscle into the blood. Fatal shock develops in rats so treated if the muscles of more than one leg and thigh have been occluded for three and a half hours or if the occlusion of only one thigh and leg persisted for six hours before release. This type of shock is definitely not

due to adenosine triphosphate washed out of the muscle because adenosine triphosphate is destroyed during the occlusion and its decomposition products appear to be relatively nontoxic. In rats surviving release after occlusion of one leg for four hours there is almost complete necrosis of the injured muscle but sufficient cells remain alive to restore themselves. After four to six weeks there is restoration of function of the leg, although the original size of the muscle bundles is not completely restored.

REFERENCES

- (1) MOON, V. H. Shock: Its dynamics, occurrence and management. Philadelphia, Lea and Febiger, 1942, 324 pp.
- (2) KENDRICK, D. B., JR., H. E. ESSEX AND H. E. HELMHOLZ, JR. *Surgery* 7:753, 1940.
- (3) GREEN, H. N. *Lancet* 2: 147, 1943.
- (4) BIELSCHOWSKY, M. AND H. N. GREEN. *Lancet* 2: 153, 1943.
- (5) BUELL, M. V., M. B. STRAUSS AND E. C. ANDRUS. *J. Biol. Chem.* 98: 645, 1932.
- (6) FLOCK, E. V., D. J. INGLE AND J. L. BOLLMAN. *J. Biol. Chem.* 129: 99, 1939.
- (7) BOLLMAN, J. L. AND E. V. FLOCK. *J. Biol. Chem.* 147: 155, 1943.
- (8) FLOCK, E. V. AND J. L. BOLLMAN. *J. Biol. Chem.* 152: 371, 1944.
- (9) EGGLETON, G. P. AND P. EGGLETON. *J. Physiol.* 68: 193, 1929.
- (10) FISKE, C. H. AND Y. SUBBAROW. *J. Biol. Chem.* 66: 375, 1925.
- (11) BYWATERS, E. G. L. *J. A. M. A.* 124: 1103, 1944.

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STUDIES ON MECHANISMS INVOLVED IN SHOCK AND ITS THERAPY

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The extensive studies of many investigators agree that the summation of the varying changes encountered in shock, as this follows different inciting agencies, is the effectiveness of the circulating blood volume (1, 2, 3). This in its turn is referred more and more to the oxygen offered to various organs by the circulating blood. The opinion has been expressed that disturbances in metabolism resulting from insufficient oxygen through inadequate circulation are important in the so-called irreversible state. Inhibition and block in the progressive formation of essential intermediates in the chain of energy producing transformations are now known (4, 5, 6), but their importance cannot yet be evaluated. The nervous system with its well known susceptibility to anoxia is definitely concerned in this problem, and requires more consideration than it has been accorded.

The importance of vasospasm has been pointed out repeatedly (7, 8). Its prompt appearance in tourniquet shock as distinct from the delay in shock from graded hemorrhage, its association with other manifestations especially hyperglycemia (9), its relation to the reactivity of the nervous system and its value as an index of the effectiveness of therapy all will be dealt with in the report that follows:

Sodium Succinate Therapy in Traumatic Shock. An earlier report (10) included detail of an adequately standardized method of producing shock by the application of tourniquets to the hind legs of dogs, and also the results of infusion of several different blood substitutes. Dog plasma proved most efficacious with recovery of 80 per cent of the animals; succinate reinforced saline was second with 43 per cent recovery.

The prompt increase in blood pressure and also the alteration in the state of the animals from coma when the infusion was started to alertness within a few hours, were impressive with both plasma and succinate reinforced saline. These favorable signs were also evident with preliminary trials of fumarate reinforced saline.

¹ The work described in this paper was done with the aid of grants from the Commonwealth Fund and under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Yale University.

In subsequent experiments designed to determine the value of repeated infusion as distinct from a single one, mixtures of sodium succinate and fumarate were used. The concentration varied from 0.8 per cent to 1.2 per cent and exceptionally as high as 2.3 per cent. The first treatment approximated 4 per cent of the animal's weight, later ones, usually after 4 and 8 hours, each were about one-fourth as large. The quantity of the two salts used for a single animal varied between 4 and 6 grams. The first 100 cc. of infusate was delivered rapidly, 20 cc. per minute; the speed was then reduced to 7 cc. per minute and this rate was used in later treatment. Therapy was started when the condition of the animal as previously reported was one of marked shock. Lack of alertness and inability to sustain the circulation as evidenced by falling pressure and cold extremities were the indications for the second and third infusions. Nine of fifteen animals survived. No significance could be attributed to the variations in the concentration and total quantity of succinate and fumarate used.

The explanation of the apparent therapeutic value of intermediates like succinate is not at hand. *In vitro* studies concerned with the influence of succinate upon vital enzymatic activities are not sufficiently in agreement at present to allow of definite conclusions. Shorr (11) was unable to demonstrate beneficial effect with succinate oxidation upon urea production in liver slices, deamination in kidney slices or phosphorylation in similar preparations of heart muscle. An explanation for this lack of utilization of energy is offered by Meyer and Potter (12) who suggest that it may be a manifestation of *in vitro* conditions.

The beneficial effects of sodium succinate therapy observed clinically have been attributed to its alkali content and consequent neutralization of acidosis. Soskin is of the opinion that acidosis is a significant factor in the development of the irreversible phase of shock (13). Others have not observed the development of significant acidosis in clinical shock during the first hour or two (14). This has been confirmed in the experiments now to be briefly recorded.

Acidosis was measured just before institution of therapy when the fifteen animals above referred were at a critical state. The highest and lowest values of the nine survivors were 49.7 vol. per cent and 23.6 vol. per cent respectively, for the six that did not recover 31.1 vol. per cent and 26.4 vol. per cent. This indicates that the degree of acidosis present when infusion was started had no relation to recovery. Moreover it was not possible to demonstrate association between the observed mild acidosis and survival in shock after hemorrhage, produced as described later in this report; nor was the correction of the acidosis by infusion with sodium succinate determining for the outcome of the experiment. Some animals died even though the carbon dioxide combining power of their blood had been returned to normal with infusion and others survived with persistence of the mild acidosis. These results are in agreement with those of Fine who found correction of acidosis by infusion with sodium bicarbonate to be without significant effect (15).

It may be concluded that development and correction of acidosis was not a major factor in the result of therapy. This should not be interpreted to mean that under other circumstances acidosis may not be of greater importance.

Vasoconstriction as Manifested by Different Methods of Recording Blood Pressure. In the experiments with repeated therapy in tourniquet shock referred to above, it was desirable to record the blood pressure at intervals over many hours. Accordingly the modified Friedman oscillograph (16, 17) was selected rather than the cannula and mercury manometer. In every instance recording with the oscillograph fell from normal to zero shortly before infusion was started. In a few animals the pressure was measured simultaneously from the femoral artery with a large gauge needle and a mercury manometer and was found to be between 60 and 70 mm. Hg at the time when the oscillograph no longer recorded.

These observations led to detailed comparison of the readings as determined by the two methods during the development of shock, following the release of tourniquets and the subsequent period of therapy. The latter consisted of infusion either of dog plasma, dog plasma albumin or normal saline, and several animals for each one of these agents. As shock developed, the pressure dropped as measured with the mercury manometer to the well known levels found in this method of its induction. The oscillogram readings at the same height as those obtained with the mercury manometer when the tourniquets were released, fell more rapidly and reached zero before therapy was instituted. The results of the latter were in sharp contrast depending on the material infused. With plasma and ultimate recovery of the animal, the oscillograph reading closely approached the level of the rising mercury manometer pressure within an hour. With albumin (4.7 per cent) or saline temporary rise in manometrically determined pressure was followed by similarly increased oscillogram readings but the discrepancy between the two persisted until the final drop of pressure preceding the death of the dog.

These findings are illustrated in the two following curves.

The discrepancy in the readings as ascertained by the oscillograph and mercury manometer may be attributed to two well known factors. The first is decreased cardiac output and pulse volume; the second, constriction involving not only arteries of the size observable in the fundus of the eye but also larger ones like the carotids and femorals. The more promptly the difference between the two methods of recording becomes manifest, and this may occur within a few minutes after the release of the tourniquets, the higher is the manometric reading likely to be and the more marked the vasoconstriction. Under these circumstances the discrepancy must be largely an expression of vasoconstriction.

Anatomical Changes. Lesions of the heart muscle were found constantly in these experiments. How far vasoconstriction was responsible for their occurrence as distinct from other factors associated with the ineffective circulating blood volume cannot be determined. They were typical of inadequate oxidation (18) and quite indistinguishable from similar change found in protracted or pernicious anemia in man. Their occurrence is readily understood on the basis of the functional studies of Meek, Eyster, etc. (19).

These lesions, encountered when dogs survived the release of tourniquets for more than 8 hours, involved particularly the papillary muscles of the left ventricle and to a lesser extent the septum. Grossly they varied in size, were pale, and

contrasting with the normal red muscle, mottled the surfaces. With sudan their content of small fat droplets was easily shown, even when the striations of the muscle fibers were well preserved and the nuclei unaltered. These lesions undoubtedly were the precursors of miliary foci in the papillary muscles found when animals that have recovered after therapy were allowed to survive 3 to 8 days. The latter were both smaller and fewer in number and represented necrotic muscle impregnated by calcium and surrounded by monocytes. Examination of the adrenal cortex of a large number of these animals consistently showed depletion of lipid of the outer two layers. The significance of the change is not known² (20).

Mention should be made of the marked reduction in the clotting time of the blood following immediately on release of the tourniquets. This has been re-

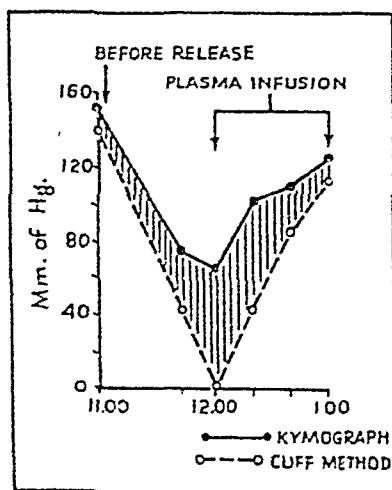


Fig. 1

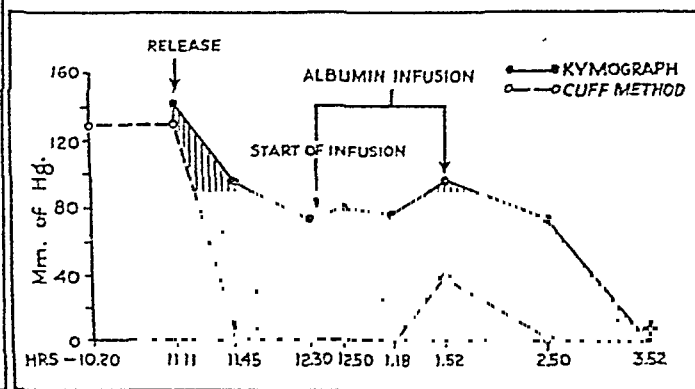


Fig. 2

Fig. 1. Dog 16. Comparison of blood pressure changes as recorded by the mercury manometer and values recorded by the Friedman oscillograph. Release of tourniquets at 11:00 a.m. Start of plasma infusions at 12:00 noon.

Fig. 2. Dog 17. Comparison of blood pressure changes as recorded by the mercury manometer and values recorded by the Friedman oscillograph. Release of tourniquets at 11:11 a.m. Start of albumin infusion 12:30 p.m.

ported (21) but deserves further attention because it became much more evident during the hot summer months. Indeed, intravascular clots complicated a considerable number of experiments. Acute death after release of the tourniquets was observed; more frequently the clot became dislodged with improved circulation after therapy and located in the pulmonary artery with the usual typical fatal result.

Hemorrhagic Shock. The first problem involved the determination of the optimal rate for the withdrawal of blood to induce symptoms comparable to those observed after the release of tourniquets.

Slow withdrawal of blood. The repeated withdrawal of blood in quantities

* Dr. Rolf Katzenstein's help in making these anatomical studies is gratefully acknowledged.

ranging from $\frac{1}{2}$ per cent to 1 per cent of the weight of the animal, up to a total of $3\frac{1}{2}$ per cent in not less than $3\frac{1}{2}$ to 4 hours, did not result in shock, nor did this follow with subsequent repeated bleedings at the same rate up to even more than 5 per cent. Hypotension was marked; hemodilution was considerable, indicating that the ingress of fluid from the tissues approximated in amount the withdrawal of blood. It would seem that the effective circulating blood volume was maintained under these conditions and that as long as the number of red blood cells was adequate to supply the minimal oxygen carrying requirements, no serious complication resulted.

Under these circumstances vasoconstriction did not occur; there was no discrepancy between the blood pressure as measured by the cuff technique and that secured from a cannula in the femoral artery. The cold, grey mucous membranes

TABLE 1

Dog 18, 9.0 kgm. Slow bleeding without development of vasoconstriction

TIME	TOTAL BLOOD WITH-DRAWN	CUFF PRESSURE	BLOOD SUGAR		HEMATO-CRIT	O ₂ IN BLOOD		REMARKS
			Carotid artery	Jugular vein		Carotid artery	Jugular vein	
	% body wt.	mm. Hg	mgm. %	mgm. %	%	vol. %	vol. %	
8:00	1.0		105	93	41.0	17.1	13.7	Animal alert throughout experiment
9:00	2.0	100			45.0			
9:30	2.25		122	115	42.0	15.4	11.1	
10:00	2.5	110						
10:45	3.0	108			35.0			Mucous membranes remained warm
11:30	3.5	100	133	119	36.0	14.1	6.7	
12:15	4.0	106						Vasoconstriction did not develop
1:15	4.5	98	142	137	34.0	13.5	6.5	
1:45	5.0	84						Dog survived without therapy
2:15	5.5	75	169	146		12.5	4.2	
3:00			182	162		12.5	5.3	

so characteristic of traumatic shock did not appear; the evidence of central nervous system involvement was minimal and at no time was there delay in securing adequate quantities of blood from the jugular veins. Its carbon dioxide value, determined in only a few experiments, was decreased, but did not reach the low levels reported (22). The oxygen of the jugular vein decreased but this was not extreme. The blood sugar was moderately increased.

These facts are illustrated in the above record, representative of a group of 20 animals.

The group of animals represented by the foregoing example, it was realized, was not in shock (23). Their condition is well described as post-hemorrhagic hypotension, usually a reversible non-fatal state if not too extreme or too prolonged.

More rapid withdrawal of blood. As the intervals between the withdrawal of

blood were reduced, the clinical picture of shock tended to become increasingly evident. This is illustrated by the following dogs representative of groups of 10.

Dog 19. The withdrawal of blood equal to 40 cc./kilo body weight in $3\frac{1}{2}$ hours resulted in hypotension and hemodilution as in the previous group. Pale, cold mucous membranes were noted at the end of $3\frac{1}{2}$ hours and this condition persisted. At 4 hours blood could be secured only with great delay from the jugular vein.

Dog 20 was bled 40 cc./kilo body weight in 2 hours and arterial constriction was observed half an hour later when the femoral artery was exposed for blood pressure determination.

Dog 21 was bled more rapidly, 40 cc./kilo body weight in 80 minutes. The hematocrit and carbon dioxide content of the blood was not much changed in this period. Arterial constriction became marked as evidenced by a drop in the jugular oxygen content and by the reduced pulse volume, which was not sufficient to work the Friedman oscillograph with the cuff pressure at a level well below the mean arterial pressure.

A type of shock quite comparable to that observed with the standard tourniquet procedure and including marked vasoconstriction resulted with few exceptions when bleeding was carried out in the following way:

1. Removal of an amount of blood equal to 10 cc./kilo body weight at 20 minute intervals until 30 cc./kilo body weight had been secured.

2. Removal of 5 cc. of blood per kilo body weight until 40 cc./kilo body weight had been withdrawn.

3. Removal of additional quantities of blood each from 2.5 to 5 cc./kilo body weight as indicated by the condition of the animal. Sixteen dogs bled in this way were not treated. They developed vasoconstriction and died within an hour after completion of the bleeding procedure.

The effort to produce shock by hemorrhage characterized by vasoconstriction and other phenomena to be described was influenced in part by experience with tourniquet shock, in part by preliminary attempts at therapy indicating that shock characterized as indicated above presented the more serious challenge.

Even though these two forms of shock, as standardized, have in common marked vasoconstriction and associated pale, cold mucous membranes, empty veins, reduced cardiac output and circulating blood volume, they contrasted with each other in the following detail.

The general condition of the animal was much worse with a considerably higher blood pressure in tourniquet shock than was the case after hemorrhage. A pressure of 70 mm. Hg for the former was serious, for the latter even 30 mm. Hg may not have been.

Hemoconcentration after tourniquet release was progressive and the packed red cell volume reached values as high as 80 per cent. After hemorrhage, hemodilution was the rule.

Hyperglycemia occurred with both procedures; but reached a much higher level after hemorrhage. In 4 experiments of traumatic shock the arterial blood sugar rose from approximately 100 mgm. per cent to 153, 167, 176 and 159 mgm.

per cent. In 50 experiments with hemorrhagic shock the blood sugar values as a rule were much higher, reaching 300 mgm. per cent and more, shortly before therapy was instituted.

The values for jugular oxygen shortly before the death of the animals subjected to traumatic shock were 2.4, 2.3, 3.9 and 3.2 vol. per cent; in hemorrhagic shock they were much lower—between 1.0 and 1.7 vol. per cent.

Further Detail Indicating the Importance of Vasoconstriction. There seems to be no absolute relation between hypotension and the onset of vasoconstriction. Dog 22 developed vasoconstriction after removal of blood corresponding to 4 per cent of his body weight in slightly less than 4 hours when the pressure was 124 mm. Hg as registered from the femoral artery with a mercury manometer and the Friedman oscillograph registered 80 mm. Hg. The appearance of vasospasm in this animal was unusual. It should be pointed out that it only occurred exceptionally with this rate of blood withdrawal.

Contrast the above with dog 23. Blood corresponding to 3.5 per cent of his body weight was withdrawn in 65 minutes and to 4 per cent in 90 minutes. The blood pressure as recorded from the femoral artery was 36 mm. Hg in 75 minutes and remained at this low level for an hour and more without vasoconstriction developing.

This animal is one of six of a total of 30 (20 per cent) that did not develop vasoconstriction even though they were bled rapidly and had a low pressure for a protracted period. The hematocrit did not vary greatly, acidosis became marked and the jugular oxygen approached what has come to be regarded as a dangerously low level. When the relation of hypotension and vasoconstriction was further analyzed, no constant association could be established. Still the trend, when bleeding was reasonably rapid, was clearly indicated in the majority. Vasoconstriction usually was observed when the pressure was between 38 and 48 mm. Hg.

It is of interest to note that vasoconstriction once established usually persisted and without therapy was followed by death. In only a few animals did the vasoconstriction disappear spontaneously.

Saline infusion after hemorrhage differed in its therapeutic effect depending upon the presence of vasoconstriction. When this was marked, only temporary improvement occurred and death almost invariably followed within a few hours.

If the vasoconstriction had not become manifest, prompt improvement in general circulation could be expected.

Dog plasma infusion in the majority of instances was followed by a permanent release of the vasoconstriction and recovery. (Vide dog 16, fig. 1 and dog 21, table 2.)

Development of Shock with Reduction of Sugar or Blood Flow to the Central Nervous System. With the standard rapid withdrawal of blood and the associated vasoconstriction, loss of responsiveness and its sequel coma usually occurred with little delay. This would be expected from the added influence of the arterial constriction to that of hypotension upon the oxygen supply to the nervous system. The reduction in oxidation resulted in a symptomatology quite similar to that of

insulin shock where the substrate is lacking without change in available oxygen. Indeed the signs of excitement followed by depression beginning with the cortex and involving progressively basal ganglia, hypothalamus and medulla as described for insulin shock (24) were almost as evident after hemorrhage when oxygen was the limiting factor.

Bearing in mind the requirements for energy production on the part of the nervous system, experiments were devised to ascertain whether preliminary reduction in available glucose or of blood supply to the nervous system would influence the induction and intensity of shock after hemorrhage.

Animals were treated with phloridzin to deplete their sugar reserve and to prevent the rise in blood sugar that normally follows the procedure involved in pro-

TABLE 2
Dog 21, 8.0 kgm. Rapid bleeding with development of vasoconstriction

TIME	TOTAL BLOOD WITH-DRAWN	CUFF PRESSURE	KYMOMOGRAPH	HEMATOCRIT	CO ₂	O ₂ IN BLOOD		REMARKS
						Carotid artery	Jugular vein	
	% body wt.	mm. Hg	mm. Hg	%	vol. %	vol. %	vol. %	
8:00	1.0	115		48	49.5	21.8	14.0	
8:20	2.0							
8:40	3.0	80		49.5	47.3	21.0	13.0	
9:00	3.5							
9:20	4.0			45.5	47.6	20.5	4.6	
9:40			34					Marked vasoconstriction
9:52			48					
10:00	4.12			41.0	38.2	20.0	5.5	
10:23	4.49		50		31.1	18.7	1.7	Infusion with fresh (citrated) plasma started
11:20			120	21.0		9.7	5.8	Plasma infusion 400 cc. completed. Dog survived

duction of shock by hemorrhage. The satisfactorily phloridzinised animals 24, 25, 26 proved to be more susceptible to hemorrhage than the untreated controls. Symptoms referable to the nervous system including loss of pupillary reactions and coma were early and prominent, even with the oxygen of the jugular vein still considerably above the danger level.

Two other animals, nos. 27, 28, were not adequately phloridzinised as their blood sugar levels rose significantly with hemorrhage. They developed the usual picture of shock with low venous oxygen. All of the five animals received saline infusion.

The outstanding fact from this series was the fatal outcome of three animals whose jugular oxygen was adequate as judged by all previous experience and the survival of the other two whose jugular oxygen was at the danger level. When it

is recalled that the blood sugar was low in the first group and high in the second, the importance of sugar is emphasized.

Ligation of the carotid arteries immediately prior to graded bleeding accentuated the neurological symptoms. Early excitement was followed rapidly by coma which persisted even when the blood pressure temporarily returned to normal after saline infusion.

One interesting observation should be recorded. Blood corresponding to 4.5 per cent of the dog's body weight was removed in $1\frac{3}{4}$ hours. The carotids were clamped when the animal was in fair condition with a blood pressure of 38 mm. Hg without vasoconstriction, and with a jugular oxygen of 3 vol. per cent.

TABLE 3

Influence of preliminary phloridzination on the outcome of hemorrhagic shock

DOG NO.	TOTAL BLOOD WITH-DRAWN	BLOOD PRESSURE LOWEST VALUES	CHANGES IN CO ₂ VALUES	BLOOD SUGAR VALUES BEFORE AND DURING HEMORRHAGE		BLOOD OXYGEN LOWEST VALUES		REMARKS
				Arterial	Venous	Arterial	Venous	
	% body wt.	mm. Hg	vol. %	mgm. %	mgm. %	vol. %	vol. %	
24	3.6	28 to 34 for 9 min.	47.1 21.1	97 125	89 105	21.1 16.8	5.5 4.1	Coma persisted when circulation was restored with saline infusion. Death 1 hr. later
25	3.5	20 to 24 for 3 min.	47.4 32.1	104 121	86 111	16.7 11.8	6.2 3.0	Coma persisted when circulation was restored with saline. Death 1 hr. later
26	5.37	28 to 36 for 3 min.	29.2 19.0	86 114	79 99	— 12.4	— 3.4	Coma. Death during infusion
27	5.0	34 to 38 for 31 min.	29.1 24.1	71 162	61 137	15.0	1.2	Not sufficiently phloridzinated. Recovery
28	4.0	36 to 30 for 2 min.	40.6 32.3	104 150	95 146	14.7	1.0	Not sufficiently phloridzinated. Recovery

Within 7 minutes the pupils, until the closure of the clamps normally reactive, became dilated and fixed. The flow was then restored in the carotids and reactivity of the pupils returned promptly. This procedure was repeated twice within $\frac{1}{2}$ hour with similar results.

This evidence of the relation of blood flow to the brain and the pupillary reactivity contributes to the interpretation of mechanism concerned in shock particularly to the significance of loss of pupillar response. It is further of interest that, by stereoscopic observation of the eye grounds, contraction of retinal arteries and also of veins was readily observed as other evidences of vasoconstriction developed after hemorrhage. The tension of the eye ball, normally more

than 20 mm. Hg, fell to 7 mm. Hg as measured by the tonometer.³ This corroborated the impression that has been gained by palpation in many experiments.

SUMMARY

1. Repeated infusion of sodium succinate and fumarate resulted in recovery from shock produced by the tourniquet method in 9 out of 15 dogs.

2. There was no indication that correction of mild acidosis, present when therapy was started, played a major rôle in recovery.

3. Vasoconstriction developed promptly and frequently was marked within 10 minutes after the release of the tourniquets. The mucous membranes became pale and cold, visible arteries were constricted and blood was only obtainable with difficulty from the collapsed veins. At this stage the blood pressure, determined by the cuff method, approached zero when the actual pressure in the femoral artery was 60 or more mm. Hg. This discrepancy disappeared with plasma infusion in contrast to a slight and temporary reduction when saline or albumen were used.

4. Structural changes found constantly in the heart muscle indicated the severity of the anoxia and suggested an explanation for the observed alterations in function of the nervous system.

5. Shock characterized by vasoconstriction followed the withdrawal of blood when this was properly graded. Saline infusion as a rule was without beneficial influence when vasoconstriction was marked and contrasted with plasma that resulted in survival of the majority of animals.

6. Functional impairment of the central nervous system associated with the anoxia resulting from hemorrhage was facilitated when the concentration of sugar was reduced by phloridzination; this was also the case with preliminary ligation of the carotid arteries.

REFERENCES

- (1) JOHNSON, G. L. AND A. BLALOCK. *Arch. Surg.* 22: 626, 1931.
- (2) PARSONS, E. AND D. B. PHEMISTER. *Surg., Gynec. and Obstet.* 51: 196, 1930.
- (3) HARKINS, H. N. *Surgery J.* 9: 231, 447, 607, 1941.
- (4) GREIG, M. E. AND W. M. GOVIER. *J. Pharmacol. and Exper. Therap.* 79: 169, 1943.
- (5) ENGEL, F. L., M. G. WINTON AND C. N. H. LONG. *J. Exper. Med.* 77: 397, 1943.
- (6) RUSSEL, J. A., C. N. H. LONG AND A. E. WILHELM. *J. Exper. Med.* 79: 23, 1944.
- (7) FREEMAN, N. E., S. A. SHAFFER, A. E. SCHACTER AND H. E. HOLLING. *J. Clin. Investigation* 17: 359, 1938.
- (8) WIGGERS, C. I. *Physiol. Rev.* 22: 93, 1942.
- (9) GALE, H. E. D. *Proc. Roy. Soc. Med.* 28: 1496, 1935.
- (10) MYLON, E., M. C. WINTERNITZ AND G. J. DE SÜTÖ-NAGY. *This Journal* 139: 313, 1943.
- (11) SHORR, E. Conference on Shock, National Research Council, Washington, D. C., December 1, 1943.
- (12) MEYER, R. K. AND V. R. POTTER. Committee on Medical Research, Subcommittee on Shock. Progress Report, December 1, 1943 and April 1, 1944.

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- (13) LEVINE, R., B. HUDDLESTON, H. PERSKY AND S. SOSKIN. Committee on Medical Research, Sub-committee on Shock. Report of December 7, 1943.
- (14) Cournand, A., R. L. RILEY, E. S. BREED, H. D. LAUSON, R. P. NOOLE, G. L. DUNCAN, D. W. RICHARDS, JR. AND M. I. GREGERSEN. Committee on Medical Research, Sub-committee on Shock, report of July 23, 1943.
- (15) FINE, J. Committee on Medical Research, Sub-committee on Shock. Report of February 1, 1944.
- (16) FRIEDMAN, I., L. H. OTT AND A. W. OUGHTERSEN. *Am. Heart J.* **16**: 575, 1938.
- (17) WATERS, L. L. *Yale J. Biol. and Med.* **12**: 441, 1939.
- (18) MOON, V. H. *Shock and related capillary phenomena*. Oxford University Press, New York, 1938.
- (19) MEEK, W. J., J. A. E. EYSTER, A. E. GILSON AND Q. R. MURPHY. Committee on Medical Research, Sub-Committee on Shock. Final report of October 26, 1943.
- (20) INGLE, D. J. *Endocrinology* **31**: 420, 1942.
- (21) MYLON, E., M. C. WINTERNITZ, R. KATZENSTEIN AND G. J. DE SÜTÖ-NAGY. *This Journal* **137**: 280, 1942.
- (22) SOSKIN, S. Conference on Shock, National Research Council, New Haven, Connecticut, July 10, 1943.
- (23) SWINGLE, W. W., J. J. PFIFFNER, H. M. VARS AND W. M. PARKINS. *This Journal* **107**: 259, 1934.
- (24) HIMWICH, H. E., J. P. FROSTIG, I. F. FAZEKAS AND Z. HADIDIAN. *Am. J. Psych.* **96**: 371, 1939.

FURTHER OBSERVATIONS ON FACTORS INFLUENCING HYPOXIC RESISTANCE OF MICE¹

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Within the last decade much attention has been focussed on anoxia and its related physiological problems. Factors influencing resistance to anoxia especially have received attention and numerous reports have been published purporting to show how such influences as age, rate of decompression, temperature, drugs, and even special diets might affect the animal's anoxic resistance.

In our laboratory we have investigated certain factors such as rate of decompression, carbon dioxide, starvation, carrot diet, dehydration, and temperature of the surrounding air, some of the results of which we wish to present:

1. *Rate of decompression on anoxic survival.* This field has been fairly well investigated since the outbreak of the war and some pertinent data have been withheld for military secrecy. It is often stated that survival is greatest with rapid decompression (1, 2). Such may be true for certain animals but not for mice. Mice (rats) survive better with slow decompression (3, 4, 5, 6, 7), apparently due to an acclimatization effect of some sort which may be the loss of body heat during the panting and sweating which usually occurs with decompression, or it may be loss of carbon dioxide, or dehydration. We have tried various rates of decompression and have found the optimum for greatest survival to be equivalent to 674 feet per minute (average). Faster or slower than this rate was less effective. Table 1 shows the results of 4 different rates of decompression. In these experiments the temperature of the air in the decompression jar was $26 \pm 1^{\circ}\text{C}$. After the start of each decompression experiment the temperature inside the animal chamber rose slightly indicating loss of heat from the mice. That fall in body temperature is significant in anoxic survival has been shown previously (8). Prolonging the rate of decompression excessively probably causes exhaustion of the mice.

2. *Carbon dioxide.* In any decompression apparatus unless a relatively great stream of air is being drawn through carbon dioxide is likely to accumulate. That carbon dioxide may influence hypoxic survival was considered a possibility. To determine in which direction carbon dioxide influences survival a comparison was made with 51 mice, 26 of which were decompressed over a 2 inch layer of soda-lime, being separated from it only by the thickness of a fine mesh cloth screen. Air previously washed free of carbon dioxide was then drawn through the decompression chamber. Twenty-five mice were used under conditions exactly the same except for the absence of the soda-lime. The results are shown in table 2. Statistically the results are not significant;

¹ Aided by a grant from the Purdue Research Foundation.

however, they show that carbon dioxide does not increase hypoxic resistance which corroborates the findings of certain others (9, 10, 11).

3. *Inanition.* The effect of starvation on anoxic sensitivity has been investigated (12, 13). In our laboratory the effects of starvation for different periods during which mice were supplied with water ad libitum was determined. In no case did inanition increase anoxic resistance (see table 3).

4. *Carrot diet.* The interesting observations of Campbell (14, 15) that protection against acute anoxia was afforded by carrots has led others to reinvestigate the problem. Some (16) have confirmed these findings using a large number of animals, others (17) employing a different technique obtained negative results. Our results agree with those of Campbell. Our mice were placed on a diet of carrots and water ad libitum for 10 days. It was noted that they consumed more water than usual and that the feces were very large and soggy indicating an excessive loss of water and an attempt to repay it by excessive

TABLE 1

NUMBER OF MICE	RATE OF DECOMP. (AVE.)	AVE. SURVIVAL PRESS.	EQUIV. ALT.
	<i>ft./min.</i>	<i>mm. Hg</i>	<i>feet</i>
44	9,384	215.8	30,967
47	3,754	179.0	34,965
61	674	152.2	38,362
30	430	174.2	35,535

TABLE 2

NUMBER OF MICE	TEMP.	RATE OF DECOMP. (AVE.)	AVE. SURVIVAL PRESS.
	<i>°C.</i>	<i>ft./min.</i>	<i>mm./Hg</i>
26 over soda-lime.....	24°	674	147.5
25 controls.....	24°	674	151.1

imbibition. When subjected to decompression these mice exhibited a noticeable absence of sweating and fewer convulsions. The possibility of NaCl loss as a result of excessive body water loss appeared and as a result other mice were placed on an exclusive carrot diet plus 0.2 per cent NaCl ad libitum for 10 days. As a result very much less water (0.2 per cent NaCl solution) was drunk. Also the loss in body weight during the feeding period was greater than with the former group given water ad libitum. When decompressed this latter group showed a significantly greater hypoxic tolerance than did the former group. Table 4 summarizes the results of the carrot diet. It might be added that the carrot-fed mice gradually assumed an orange color due to the carotinoid pigments present indicating considerable assimilation of these substances by the tissues. It should be added that at the same time these experiments were being conducted with mice others were in progress relative to factors influencing the anoxic survival of the decapitated rat head. Using

a modification of Selle's (18) method the survival of the decapitated heads of 10 rats fed exclusively carrots and water for 10 days was compared with survival of control rats of the same age and weight. The results showed that the carrot diet did not increase the survival time of the decapitated rat head as based upon the total gasping time or total number of gasps. Evidently then carrots do not affect the anoxic resistance of the respiratory center.

5. *Dehydration.* Because of the great loss of weight in mice fed exclusively on carrots and the probability that most of the weight lost was water the problem then arose of determining the relationship of dehydration to hypoxic survival. This was done in two different ways. The first consisted of exposing mice water-starved for various periods of time to gradually decreasing barometric pressure until dead, which was the routine used in the previous experiments. The second method consisted of quickly decompressing to 190 mm. Hg pressure

TABLE 3

NUMBER OF MICE	DURATION OF INANITION	RATE OF DECOMP. (AVE.)	AVE. SURVIVAL PRESS.
	<i>hrs.</i>	<i>ft./min.</i>	<i>mm. Hg</i>
61 controls		674	152.2
16	24	674	164.1
16	48	674	169.2
16	72	674	176.7

TABLE 4

NUMBER OF MICE	DIET	AVE. WT. LOSS	RATE OF DECOMP. (AVE.)	AVE. SURVIVAL PRESS	EQUIV. ALT.
		%	<i>ft./min.</i>	<i>mm. Hg</i>	<i>feet</i>
61	controls		674	152.2	38,362
21	carrots plus H ₂ O	13.2	674	138.2	40,382
24	carrots plus NaCl	18	674	129.3	41,776

(requiring 5 min. to reach this pressure) and then quickly recompressing to room pressure in the following 3 minutes, and determining the percentage of mice surviving. In this latter method equal numbers of water starved and control mice were used simultaneously in the same chamber so that each experiment was self-controlled. Data for the first (A) and second (B) methods are given in table 5. It is apparent then that water starvation greatly enhances the hypoxic resistance in mice and may be the explanation at least in part of the beneficial effect shown by carrot diet. Comparison of A and B in table 5 indicates a discrepancy in that 36 hours of water starvation in A represents the optimum period while 48 hours appears to be optimum in B. The passage of time alone does not necessarily indicate the amount of water loss. Air temperature and humidity and possibly other factors are also important here. The results show however that water loss is of cardinal importance in hypoxic resistance and with the exception of temperature is more influential than any

other ordinary factor in adult mice. This fact may explain why a slow rate of decompression favors hypoxic resistance inasmuch as more time is allowed for evaporation of water by panting and sweating as well as for concurrent fall of body temperature.

TABLE 5

A. (Slow decompression at rate of 674 feet per minute)

NUMBER OF MICE	DURATION OF H ₂ O STARVATION	AVE. WT. LOSS	AVE. SURVIVAL PRESS	EQUIV. ALT.
	<i>hrs.</i>	<i>%</i>	<i>mm. Hg</i>	<i>feet</i>
61	controls	0	152.2	38,362
16	12		131.2	41,470
16	24	18.1	120.6	43,235
20	36	19.5	101.4	46,866
20	48	21.7	111.5	44,878

B. (Quick decompression and recompression)

DURATION OF H ₂ O STARVATION	NUMBER OF MICE	NUMBER SURVIVING	% SURVIVING	DIFFERENCE IN % (A - B)
<i>hrs.</i>				
24	30	12	A. 40.0	3.4
controls	30	11	B. 36.6	
36	32	10	A. 31.2	10.4
controls	32	6	B. 18.8	
48	30	12	A. 40.0	13.4
controls	30	8	B. 26.6	

TABLE 6

NUMBER OF MICE	AIR TEMP.	RATE OF DECOMP. (AVE.)	AVE. SURVIVAL PRESS.	EQUIV. ALT.
	<i>°C.</i>	<i>ft./min.</i>	<i>mm. Hg</i>	<i>feet</i>
16	29.5	674	179.6	34,895
61	26	674	152.2	38,362
20	20	674	121.2	43,131
16	10	674	91.6	48,994

6. *Temperature.* It has been shown that reduction of oxygen tension (barometric pressure) lowers body temperature (8, 19, 20). Thus the animals tend to become poikilothermic when subjected to decompression. This is facilitated by lowering the temperature of the surrounding air. Table 6 illustrates nicely the influence of air temperature on anoxic survival. The temperature is that of the start of the experiment. The explanation of the physiological effect of temperature is doubtless the rate of oxygen consumption which is lowered by lowering body temperature.

SUMMARY

The effects of such factors as rate of barometric decompression, carbon dioxide, starvation, carrot diet, dehydration, and air temperature on hypoxic survival of mice have been investigated. Our results have demonstrated the following facts, some of which are corroboration of earlier work, others of which are unique.

1. Mice tolerate hypoxia best if decompressed slowly (approximately 674 feet per second as an average). Prolonging the rate too greatly results in earlier failure of the mice.

2. Carbon dioxide has no significant effect on hypoxic survival not being beneficial to greater tolerance.

3. Inanition decreases hypoxic resistance in direct proportion to the duration of starvation.

4. An exclusive diet of carrots for 10 days increases the resistance of mice to anoxia which is apparently related to water loss from the tissues.

5. Dehydration up to approximately 20 per cent of total body weight significantly increases hypoxic resistance, beyond 20 per cent diminishes resistance.

6. Reduction of the temperature of the surrounding air increases hypoxic resistance in direct proportion as the air temperature is lowered.

REFERENCES

- (1) ARMSTRONG, H. G. Principles and practices of aviation medicine. Baltimore, Williams & Wilkins Co., 1939.
- (2) ARMSTRONG, H. G. AND J. W. HEIM. *J. Aviat. Med.* 9: 45, 1938.
- (3) STULLKEN, D. E. AND W. A. HIESTAND. *Proc. Soc. Exper. Biol. and Med.* 54: 260, 1913.
- (4) HAILMAN, H. F. *Proc. Soc. Exper. Biol. and Med.* 53: 221, 1913.
- (5) HIESTAND, W. A. AND H. M. ROGERS. *Anat. Rec.* 87: 21, 1913.
- (6) RUFF, S. AND H. STRUGHOLD. *Compendium of aviation medicine*. Alien Property Custodian. 1912.
- (7) EMERSON, G. A. AND E. J. VAN LIERE. *J. Lab. and Clin. Med.* 28: 689, 1913.
- (8) GELLHORN, E. *This Journal* 120: 190, 1937.
- (9) JOHNSON, A. E., M. ECKMAN, C. RAMSEY, JR. AND A. L. BARACH. *J. Aviat. Med.* 13: 130, 1912.
- (10) HINWICH, H., J. FAZEKAS, H. HERLICK, A. E. JOHNSON AND A. L. BARACH. *J. Aviat. Med.* 13: 177, 1912.
- (11) YOUNG, W. B., M. PENNINGTON, H. E. GROSWOLD, JR. AND J. A. GIUS. *Proc. Soc. Exper. Biol. and Med.* 52: 320, 1913.
- (12) LEBLOND, C. P., J. GROSS AND H. LAUGIER. *J. Aviat. Med.* 14: 262, 1913.
- (13) LAUGIER, H. AND C. P. LEBLOND. *Rev. Canad. de Biol.* 2: 713, 1913.
- (14) CAMPBELL, J. A. *Quart. J. Exper. Physiol.* 28: 231, 1938.
- (15) CAMPBELL, J. A. *Quart. J. Exper. Physiol.* 29: 259, 1939.
- (16) NELSON, D., S. GOETZL, S. ROBINS AND A. C. IVY. *Proc. Soc. Exper. Biol. and Med.* 52: 1, 1913.
- (17) WITZIG, P. AND F. E. D'AMOUR. *This Journal* 140: 301, 1913.
- (18) SELLE, W. A. *Proc. Soc. Exper. Biol. and Med.* 51: 50, 1912.
- (19) BEHAGUE, P., M. GARBAUX AND C. RICHET. *Compt. rend. Soc. Biol.* 96: 766, 1927.
- (20) CHEVILLARD, L. AND A. MAYER. *Ann. de Physiol.* 11: 255, 1935.

A COMPARISON OF THE RENOTROPHIC¹ WITH THE ANDROGENIC ACTIVITY OF VARIOUS STEROIDS²

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It has been demonstrated that androgens increase the size of the kidneys of the mouse (2, 3, 4, 5), rat (6, 7, 8) and dog (9). The greatest and most uniform changes are noted in the mouse (4). Significant increases in the rat kidney occur only after hypophysectomy (7) or on injecting an optimum dose of testosterone propionate (8). The small (10–20 per cent) increase in the kidney weight of the normal rat after testosterone injections probably is not significant⁴ (10).

Since the kidney is an important metabolic organ, it is very probable that the change in its size after testosterone propionate injections is a reflection of the metabolic demands imposed upon the body by the androgen (12, 13, 14). It seemed, therefore, that a study of the available steroids for both their renotrophic and androgenic properties might provide a valuable "screening process" for the selection of steroids with greater renotrophic (metabolic) than androgenic effects. That such a compound(s) exists was suggested by the fact that urinary androgenic extracts possess very marked protein anabolic properties (15) yet these preparations contain no testosterone.

METHODS. *Pellets.* The steroids⁵ were made into cylindrical pellets of 14 ± 1 mgm., diameter 2.7 mm. and length 2.7 ± 0.1 mm., by means of a hand press designed in this laboratory.

¹ This laboratory has adopted the suffix -trophic in preference to -tropic in accordance with the suggestion of Corner (1).

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³ The author is indebted to his wife, Irene Kochakian, for her valuable technical assistance in this investigation.

⁴ Treatment of female and castrated male rats for short and long periods of time with small and multiple or large pellets of testosterone or testosterone propionate has had no effect on kidney size. In one series of experiments the pellet was implanted directly in the kidney without any effect. There is, however, a definite increase in the arginase content of the kidneys of the treated rats (11, unpublished).

⁵ The author is indebted to Dr. E. C. Kendall for the 11-dehydrocorticosterone; Dr. J. J. Pflüger for the 17-hydroxy, 11-dehydrocorticosterone; Drs. K. Dobriner and S. Liebermann for the etiocholanol-3 α , one-17; pregnanol-3 α , one-20 and allopregnanol-3 α , one-20; Dr. M. Ehrenstein for the androstenediol-3 β , 5, dione-6, 17; and to Drs. E. Oppenheimer and C. R. Scholz for all of the other steroids, many of which were prepared specially for this and related studies by Dr. C. R. Scholz.

*Animals*⁵. Mice of the highly inbred Murray-Little dba strain were castrated under ether anesthesia at 16.0 to 19.5 grams body weight. They were fed Purina Fox Chow checkers.

In most of the experiments four or more mice were used. In a few instances, however, the scarcity of material limited the number of animals but only obvious conclusions are made in these cases.

Duration of experiments. The steroid pellets were implanted (4) thirty days after castration and were allowed to remain *in situ* for thirty days. Many of the more active compounds also were tested over a period of ten days. Testosterone and desoxycorticosterone also were tested in a twenty-day series of experiments.

Autopsy. The mice were fasted twenty-four hours before autopsy and were killed by decapitation. The organs were immediately removed and weighed on a Roller-Smith torsion balance. The pellet was removed, washed in distilled water, dried in a desiccator over calcium chloride and reweighed.

RESULTS. *Normal animals.* The renotrophic-androgenic condition of the normal mouse (tables 1 and 2) is most nearly simulated by androstanol-17 α ,one-3. Although this may be interpreted to indicate that this compound is identical or closely similar to that naturally occurring in the mouse, it is more likely, however, that the renotrophic-androgenic status in the organism is governed by a number of substances.

The effect of castration. In agreement with the previous report (4), castration produced a marked retardation in growth of the kidneys and seminal vesicles and prostates accompanied by hypertrophy of the thymus. There was no significant difference in the kidney weights of the castrated control mice for the 10, 20 or 30 day experiments (4).

Chemical structure and activity. There are twenty-one compounds that are able significantly to increase the weight of the kidneys of the castrated mouse (table 1). The increases are neither related to amount of steroid absorbed nor androgenic activity (cf. table 3), but do seem to be related to chemical structure.

The 17 α -hydroxy group is necessary for maximum activity of the molecule. The replacement of this radical by a ketone (androstenedione-3,17; androstenedione-3,17) greatly reduces and the introduction of a 17 β -hydroxyl completely obliterates (*cis* testosterone) all activity.

The 3 β -hydroxyl group also tends to decrease (isoandrosterone) or completely remove activity (androstanediol-3 β ,17 α), but its effect is offset somewhat by the introduction of unsaturation (dehydroisoandrosterone) or a methyl group in the 17 position (17-methyl androstanediol-3 β ,17 α and 17-methyl androstenediol-3 β ,17 α).

Saturation of the ring double bond (androstanol-17 α ,one-3) results in a decrease in total activity which is probably due in part to the much slower rate of absorption (tables 3 and 4). Furthermore, there is a greater decrease in the

⁵ All of the mice were generously provided by S. G. Warner of the Biological Station Springville, N. Y.

renotrophic than the androgenic potency. Of even greater significance is the change in the renotrophic-androgenic ratio in the saturated $3\alpha,17\alpha$ diols. Androstenediol- $3\alpha,17\alpha$ has a much greater effect on the weight of the kidney

TABLE 1

The effect of various steroid pellets on the kidney, seminal vesicles and prostate and thymus of the mouse

STERIOD	NO. OF MICE	STERIOD ABSORBED	CHANGE FROM CASTRATED CONTROLS ^a		
			Kidneys % ^b	Seminal vesicles + pros- tates % ^b	Thymus % ^b
30-day experiments					
Testosterone.....	9	8.3	108	2860	-91
Testosterone propionate ^c	11	4.4 ^k	99	2700	-94
17-Methyl androstenediol-3 α ,17 α	7	2.6 ^q	98	1380	-76
17-Methyl testosterone ^d	6	8.5 ⁱ	96	2700	-97
Androstanol-17 α , one-3.....	6	2.6	79	2390	
Androstenediol-3 α ,17 α	11	1.7	77	1245	-82
(Normal mice).....	16		61	1962	-42
17-Vinyl testosterone.....	8	8.1 ^m	53	1100	-36
Testosterone acetate 3, prop-17.....	2	1.3 ⁿ	42	1800	-79
Desoxycorticosterone acetate ^e	3	9.3	40	-9	-18
Androstenedione-3,17.....	6	10.6	32	1570	-88
17-Ethyl testosterone.....	9	5.1 ^o	32	700	-24
17-Methyl androstenediol-3 β ,17 α	5	0.4	29	-9	-12
17-Hydroxy, 11-dehydrocorticosterone ^f	2	15.1 ^p	27	-9	-100
Androstenedione-3, 17.....	7	9.2	19	327	-58
Androsterone.....	6	3.7	18	54	-46
Androstenediol-3 α ,17 α , acetate-3.....	3	0.7	17	-18	-24
17-Methynyl androstenediol-3 β ,17 α	6	0.9	17	82	-9
α -Estradiol ^g	6	2.6	15	54	-70
17-Ethynyl androstenediol-3 β ,17 α	5	0.5	13	-18	+9
Dehydroisoandrosterone.....	5	10.9	13	73	-24
17-Ethynyl androstenediol-3 β ,17 α	5	1.0	5	0	+3
17-Ethynyl testosterone ^h	6	0.5	5	0	+12
Androstenediol-3 β ,17 α	6	0.2	5	18	-15
3,17-Dimethyl androstadienol-17 α	6	2.6	4	-9	+9
Progesterone ⁱ	5	5.6	3	9	-15
Testosterone benzoate.....	6	0.2	2	27	-21
11-dehydrocorticosterone ^j	4	17.6 ^p	2	-45	-27
Androstenediol-3 β ,5, dione-6, 17.....	1	6.8	2	-45	+12
Isoandrosterone.....	8	9.3	1	18	-33
cis-Testosterone.....	4	3.6	1	18	-9
Pregnenol-3 β , one-20.....	5	0.8	0	-9	+3
3-Methyl androstadienol-17 α	6	5.7	-1	9	0
Androstenediol-3 β ,17 α	7	0.7	-2	18	-24
Androstenediol-3 β ,17 β	3	2.1	-3	-18	0
Allo-pregnanol-3 α , one-20.....	2	0.1	-5	0	-12
Androstenediol-3 α ,17 α , diacetate.....	5	0.2	-6	-18	-21
Etiocholanol-3 α , one-17.....	1	7.9	-8	+18	-6
Pregnanol-3 α , one-20.....	1	2.9	-16	-36	-18

TABLE 1—*Concluded*

STEROID	NO. OF MICE	STEROID ABSORBED	CHANGE FROM CASTRATED CONTROLS ^a		
			Kidneys % ^b	Seminal vesicles + pros- tates % ^b	Thymus % ^b
20-day experiments					
Testosterone + desoxycorticosterone.....	4	{ 7.2 13.9	137	2410	-85
Testosterone.....	2	6.0	99	2260	-79
Desoxycorticosterone.....	4	12.0	24	-9	-18
10-day experiments					
Testosterone + desoxycorticosterone.....	4	{ 3.4 8.8	86	1270	-85
Testosterone.....	9	3.3	60	1130	-61
Testosterone + α -Estradiol.....	5	{ 3.2 0.7	58	736	-61
17-Methyl testosterone.....	3	3.1	56	1090	-76
Testosterone propionate.....	5	1.7	55	1110	-79
Androstanediol-3 α ,17 α	4	0.8	46	527	-58
Androstanol-17 α ,one-3.....	4	0.7	40	1060	-58
Desoxycorticosterone.....	4	10.2	22	91	-55
α -Estradiol.....	5	0.6	20	191	-49
11-Dehydrocorticosterone.....	5	12.5	18	-18	-97

^a The average values of 28 castrated mice; kidneys 263(231-292) seminal vesicles and prostates 11(7-13); thymus 33 (23-43).

^b Per cent of averages.

^c Perandren.

^d Metrandren.

^e Percorten.

^f Pfiffner's compound F, Kendall's compound E.

^g Ovocylin.

^h Lutocylol, anhydrohydroxyprogesterone, pregnen-yn-ol-one.

ⁱ Lutocylin.

^j Kendall's compound A.

^k Equivalent to 3.7 mgm. of testosterone.

^l Equivalent to 8.1 mgm. of testosterone.

^m Equivalent to 7.5 mgm. of testosterone.

ⁿ Equivalent to 0.94 mgm. of testosterone.

^o Equivalent to 4.7 mgm. of testosterone.

^p Only traces of material remaining at autopsy (cf. fig. 1).

^q Equivalent to 2.5 mgm. androstanediol-3 α ,17 α .

than on the seminal vesicles and prostates. The introduction of a 17-methyl group further enhances the renotrophic-androgenic ratio. Somewhat similar results are obtained when a pellet of α -estradiol or desoxycorticosterone is implanted simultaneously with a pellet of testosterone.

TABLE 2
The renotrophic-androgenic ratio of various steroids

STERIOD	INCREASED KIDNEY WEIGHT/INCREASED SEMINAL VESICLE AND PROSTATE WEIGHT DURATION OF EXPERIMENT		
	30 days	20 days	10 days
17-Methyl androstane-3 α ,17 α	1.70		
Androstane-3 α ,17 α	1.48		2.09
Testosterone + α -Estradiol.....			1.89
Testosterone + Desoxycorticosterone.....		1.36	1.61
17-Vinyl Testosterone.....	1.14		
17-Ethyl Testosterone..	1.09		
Testosterone.....	0.90	1.05	1.27
Testosterone Propionate.....	0.88		1.20
17-Methyl testosterone.....	0.85		1.23
Androstanol-17 α ,one-3.....	0.79		0.90
normals	0.74		
Testosterone acetate-3,propionate-17.....	0.55		
Androstenedione-3,17.....	0.49		

TABLE 3
The relative renotrophic efficacy of steroids

STEROID	INCREASE IN WEIGHT OF KIDNEYS (MG.)			
	30 day experiments		10 day experiments	
	Per mgm. steroid	Per mole $\times 10^{-3}$ steroid	Per mgm. steroid	Per mole $\times 10^{-3}$ steroid
Androstanediol-3 α ,17 α	119.4	349.0	151.2	441.0
Testosterone acetate-3, propionate-17.....	84.5	336.5		
17-Methyl androstanediol-3 α ,17 α	99.3	302.0		
Androstanol-17 α ,one-3.....	79.6	231.0	150.0	434
17-Ethynyl androstanediol-3 β ,17 α	70.0	221.5		
Testosterone propionate.....	59.1	214.0	85.8	306
Androstanediol-3 α ,17 α ,acetate-3.....	61.5	205.5		
α -Estradiol.....	66.6	180.0	86.7	234
17-Methyl androstanediol-3 β ,17 α	47.8	145.2		
Testosterone.....	34.2	98.4	47.5	137
17-Methyl testosterone.....	31.3	94.5	47.7	144
17-Vinyl testosterone.....	17.0	53.4		
17-Ethyl testosterone.....	16.5	52.1		
Desoxycorticosterone acetate.....	11.9	44.2		
Desoxycorticosterone.....			15.6	51.4
Androsterone.....	12.5	36.1		
Androstenedione-3,17.....	8.0	22.9		
Androstanedione-3,17.....	5.4	15.6		
Dehydroisoandrosterone.....	3.0	8.6		
17-Hydroxy,11-Dehydrocorticosterone.....	1.1	4.0		
11-Dehydrocorticosterone.....			3.7	12.7

Alkyl groups. The methyl group has a variable effect on the activity of the various compounds. It neither enhances nor decreases the activity of testosterone but when present on the 17 carbon atom it definitely increases the activity of androstanediol-3 α ,17 α , androstanediol-3 β ,17 α and androstenediol-3 β ,17 α (table 1). The position of the methyl groups, moreover, plays an important rôle in its ability to increase activity. The 3-methyl group in contrast to the 17-methyl has no effect by itself (3-methyl androstadienol-17 α); furthermore when added to a compound containing a 17-methyl group (3,17-dimethyl androstadienol-17 α) it offsets the enhancing effect of the latter radical.

TABLE 4
The relative androgenic efficacy of the steroids

STERIOD	INCREASE IN WEIGHT OF THE SEMINAL VESICLES AND PROSTATES (MGM.)			
	30 day experiments		10 day experiments	
	Per gram steroid	Per mole $\times 10^{-5}$ steroid	Per gram steroid	Per mole $\times 10^{-5}$ steroid
Testosterone acetate 3,propionate-17	152.5	606.0		
Androstanol-17 α ,one-3.....	102.1	296.0	167.0	484
Testosterone propionate.....	67.5	240.0	71.6	255
Androstanediol-3 α ,17 α	80.4	235.0	72.5	212
17-Methyl androstanediol-3 α ,17 α	58.4	177.8		
Testosterone.....	37.9	109.2	37.6	108
17-Methyl testosterone	35.0	105.8	38.7	117
17-Ethyl testosterone.....	15.1	47.7		
17-Vinyl testosterone	15.0	47.0		
Androstenedione-3,17.....	16.3	46.6		
17-Methyl androstanediol-3 β ,17 α	10.0	30.4		
Androstenedione-3,17	3.9	11.3		
α -Estradiol	2.3	6.2	35.0	94
Androsterone ..	1.6	4.6		
Dehydroisoandrosterone ..	0.7	2.0		
Desoxycorticosterone			1.0	3.3

In contrast to the 17-methyl group, the 17-ethyl group greatly decreases the potency of testosterone, the 17-vinyl group also decreases the activity but to a lesser degree than the ethyl group, and the 17-ethynyl group completely removes all activity. The decrease in activity may be due in part to the lower rate of absorption (tables 3, 4) as a result of addition of these groups but some of the change must be due to the chemical nature of the alkyl groups. There is almost as much 17-vinyl testosterone as testosterone or 17-methyl testosterone and about twice as much as testosterone propionate absorbed. Also the amount of ethyl testosterone absorbed, though less than that of testosterone or 17-methyl testosterone, is greater than that of testosterone propionate.

The decrease in renotrophic activity for both 17-ethyl and 17-vinyl testosterone is somewhat less than the decrease in androgenic activity. Consequently

these two compounds have a greater renotrophic-androgenic ratio than testosterone.

Testosterone and derivatives. Testosterone, testosterone propionate and methyl testosterone exhibit the greatest effect on both kidney and seminal vesicle and prostate weight.

Esterification of testosterone results in a greater efficacy of the material (tables 3 and 4), but when the procedure results in too great a decrease in the rate of absorption of the hormone (testosterone benzoate) then the physiological activity also disappears. Furthermore, there is a decided change in the renotrophic-androgenic ratio in the animals treated with the diester of testosterone. This change may be due to either the decreased amount of material absorbed or the diester *per se*.

Urinary hydroxy ketosteroids. The hydroxy ketosteroids known to occur in urine show no, or slight, activity in both renotrophic and androgenic activities. Experiments with a crystalline fraction containing a mixture of all of the α -hydroxy ketones present in normal male urine⁷ also did not exhibit any activity.

Ovarian and related steroids. Neither progesterone nor its related compounds including the synthetic product, anhydrohydroxyprogesterone (17-ethynyl testosterone) affect any of the organs studied. The one estrogen, α -Estradiol, investigated however showed significant effects on both the kidneys and the seminal vesicles and prostates. Furthermore, when the estrogen is implanted simultaneously with testosterone, it causes a decrease in the androgenic activity of this substance.

Adrenal cortical steroids. All of the adrenal cortical steroids⁸ show variable but significant renotrophic activity. The failure of 11-dehydrocorticosterone to show any renotrophic activity in the 30-day tests probably is due to the rapid rate of absorption of this material (fig. 1). A somewhat similar effect is noted with desoxycorticosterone; this compound produces a small but definite increase of the sex organs and a decrease in the size of the thymus for the 10-day but not for the 20-day experiment. Furthermore, the longer period of treatment does not cause a further increase in kidney weight. Apparently, large doses of this material are necessary.

The implantation of a pellet of desoxycorticosterone simultaneously with that of testosterone results in summation of all of the activities of the two compounds (cf. 7) with a resulting increase in the renotrophic-androgenic ratio.

Efficacy of the respective steroids. Since all of the steroids were implanted as pellets of the same size and weight, the amount of material available was governed by the rate of absorption of the several compounds. Therefore, the increase in renotrophic (table 3) and androgenic (table 4) activities has been calculated as per milligram and mole $\times 10^{-5}$ of steroid. The compounds are listed in decreasing order according to the values obtained on the mole basis.

⁷ Kochakian, unpublished.

⁸ A crystalline mixture (M. H. Kuizenga, Upjohn) containing chiefly 17-hydroxy, 11-dehydrocorticosterone and 17-hydroxycorticosterone also showed small and variable but definite renotrophic activity.

The renotrophic efficacy of the steroids is greatest in the 10-day experiments. The degree of difference between the two periods of treatment, however, is not the same for the various compounds. The androgenic efficacy of the short term experiments, on the other hand, decreases slightly, does not change or increases markedly over that of the experiments of long duration.

The much greater efficacy of the esters of testosterone, especially the diester, suggests that the amount of material absorbed even from the monoester is much more than necessary to bring about a maximum rate of response⁹ in the organs. Thus the relative efficacies are true only for the conditions of the experiments.

Thymus. The decrease in thymus weight in general is proportional to the increase in androgenic or estrogenic activity with the exception of the adrenal cortical compounds. The effectiveness of the latter steroids is: 17-hydroxy, 11-dehydrocorticosterone, 11-dehydrocorticosterone, and finally, desoxycorticosterone (cf. 16). Furthermore, the amount of available material present definitely influences the degree of regression of the thymus (fig. 1).

The regression of the thymus after administration of androgens and estrogens is well known.

DISCUSSION. Since the urinary hydroxy ketones have very little effect on the weight of the kidneys and androstanediol-3 α ,17 α has a marked and preferential effect on this organ, it may be assumed that the highly potent androgenic (17) and protein anabolic property (15) of urinary extracts is present in the hydroxy non-ketonic fraction. This portion of the extract forms a large part, 42 per cent,¹⁰ of the neutral (androgenic) extract of urine (15). Furthermore, not only are the known hydroxy ketones of urine feeble in their androgenic and renotrophic properties but also at least two of them, androsterone and dehydroisoandrosterone, possess no protein anabolic properties.¹¹

The increase in kidney size varies with the nature of the metabolic demands imposed upon the organism by the steroids. The greatest increases in kidney size are obtained with those compounds that have protein anabolic properties (cf. 12). On the other hand, only small and variable increases in the size of the kidney result after the administration of the steroids that stimulate protein catabolism (18). The increase in arginase activity of the kidney also is greater with the protein anabolic than catabolic steroids. Furthermore, the changes in arginase content of the kidneys are not related to the increase in size of the kidney but to the compound administered. Thus, although α -estradiol and desoxycorticosterone cause similar increases in kidney size, the former increases (12) and the latter decreases¹² the arginase activity of this organ. The

⁹ The implantation of two pellets of testosterone propionate instead of one in order to double the amount of material absorbed gave no further increase in kidney or seminal vesicle and prostate weight in either 10 day or 30 day experiments (Kochakian, C. D., unpublished). The arginase content of the kidneys of the doubly treated mice, however, was twice that of the singly treated animals (cf. 12).

¹⁰ Kochakian, unpublished.

¹¹ Bassett, S. H., E. H. Keutmann, and C. D. Kochakian, unpublished.

¹² Kochakian, unpublished.

effect of desoxycortico-sterone probably is due to alterations in the mineral metabolism of the body (19).

Since this investigation is concerned with a comparison of the renotrophic with the androgenic effect of the steroids, no detailed attempt is made to compare the androgenic effects of this study with those obtained in the capon (cf. 20) and rat (cf. 20) or the controversial renotrophic effects (6, 7) obtained in the

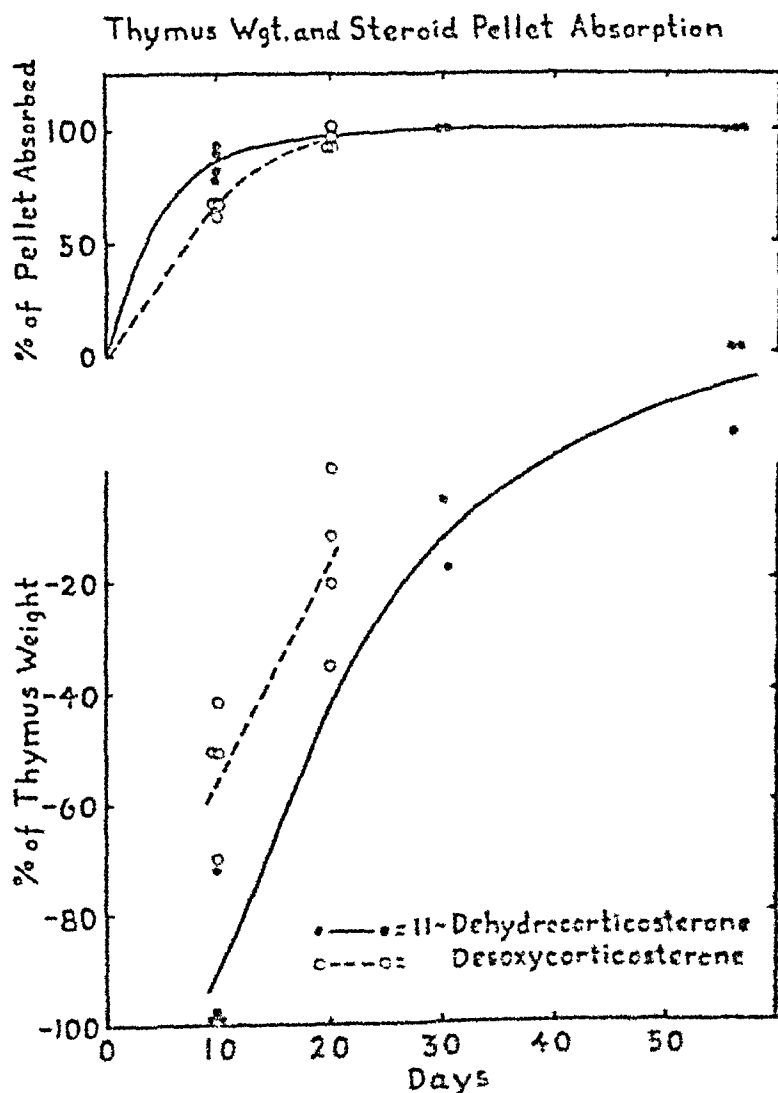


Fig. 1

rat. Suffice it to state that the response varies with species, dose and method of administration. Thus the ability of other investigators to obtain androgenic activity with androstenediol-3 β ,17 α (cf. 20), androstenediol-3 β ,17 β (cf. 20), testosterone benzoate (17, cf. 20), and renotrophic effect with progesterone (7), and the inability to obtain a renotrophic effect with testosterone (7, 21), is due to either species differences or amount of steroid administered. The investigators injected the steroids in oil solution and in many cases in enormous doses. On the other hand the results of this report are obtained

by the more efficient pellet implantation method (22, 4) which has demonstrated that only very small amounts of the more potent hormones are necessary to produce maximum physiological responses.

SUMMARY

The effect of thirty-nine steroids on the size of the kidneys, seminal vesicles and prostates and the thymus of the castrated mouse (dba strain) was studied by the subcutaneous implantation of the steroids as cylindrical pellets weighing 14 ± 1 mgm. Twenty-one of the compounds significantly increased the kidney and sixteen the seminal vesicle and prostate weights of the animals. The changes are related to the chemical structure of the substances.

A comparison of the renotrophic with the androgenic effect revealed that androstanediol- $3\alpha, 17\alpha$ and to a greater extent its 17-methyl derivative preferentially increased the kidney size to a marked degree. A similar but probably not identical effect was obtained when either an α -estradiol or desoxycorticosterone pellet was implanted simultaneously with a pellet of testosterone. A somewhat greater renotrophic-androgenic ratio was observed in the mice treated for 10 days than in those treated for 30 days.

A study of the relative efficacy of the renotrophic and androgenic effects suggested that the rate of absorption of many of the steroids, especially testosterone, 17-methyl testosterone and testosterone propionate, provided more of the hormone than was necessary for the maximal needs of the organism.

The urinary hydroxy ketones showed slight or no activity.

The thymus decreased in size in proportion to the androgenic or estrogenic activity of the steroids except for the adrenal cortical steroids. The order of potency of the latter compounds was: 17-hydroxy, 11-dehydrocorticosterone, 11-dehydrocorticosterone and desoxycorticosterone.

REFERENCES

- (1) CORNER, G. W. *Endocrinology* **33**: 405, 1913.
- (2) SELYE, H. *J. Urol.* **42**: 637, 1939.
- (3) PFEIFFER, C. A., V. M. ENNELL AND W. U. GARDNER. *Yale J. Biol. Med.*, **12**: 493, 1940.
- (4) KOCHAKIAN, C. D. *Endocrinology* **28**: 478, 1941.
- (5) CRABTREE, C. E. *Endocrinology* **29**: 197, 1941.
- (6) KORENCHEVSKY, V. AND M. A. ROSS. *Brit. Med. J.* **1**: 645, 1910.
- (7) SELYE, H. *Canadian M. A. J.* **42**: 113, 1940. *J. Urol.* **46**: 110, 1941.
- (8) LUDDEN, J. B., E. KRUGER AND I. S. WRIGHT. *Endocrinology* **28**: 619, 1941.
- (9) WELSH, C. A., A. ROSENTHAL, M. R. DUNCAN AND H. C. TAYLOR. *This Journal* **137**: 338, 1942.
- (10) SELYE, H. *Proc. Soc. Exper. Biol. and Med.* **46**: 142, 1941.
- (11) KOCHAKIAN, C. D. AND L. C. CLARK. *J. Biol. Chem.* **143**: 795, 1942.
- (12) KOCHAKIAN, C. D. *J. Biol. Chem.* In press.
- (13) KOCHAKIAN, C. D. AND R. P. FOX. *J. Biol. Chem.* **153**: 699, 1944.
- (14) CLARK, L. C., C. D. KOCHAKIAN AND R. P. FOX. *Science* **98**: 89, 1943.
- (15) KOCHAKIAN, C. D. AND J. R. MURLIN. *J. Nutrition* **10**: 437, 1935.
- (16) WELLS, B. B. AND E. C. KENDALL. *Proc. Staff Meetings Mayo Clinic* **15**: 133, 1940.
- (17) KOCHAKIAN, C. D. *Endocrinology* **22**: 181, 1938.

- (18) LONG, C. N. H., B. KATZIN AND E. G. JELLY. *Endocrinology* **26**:309, 1940.
- (19) DURLACHER, S. H., D. C. DARROW AND M. C. WINTERNITZ. *This Journal* **136**: 340, 1942.
- (20) KOCH, F. C. *Physiol. Reviews* **17**: 153, 1937.
- (21) ALBERT, S. AND H. SELYE. *J. Pharmacol. and Exper. Therap.* **75**:308, 1942.
- (22) DEANSLY, R. AND A. S. PARKES. *Proc. Roy. Soc. London (Series B)* **124**:279, 1937.

THE SITE OF METABOLISM OF PROGESTERONE IN THE RABBIT¹

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Progesterone is metabolized by the body (1, 2) and excreted in part as pregnanediol glucuronate (2, 3). The tissues responsible for this conversion are not known. It has been demonstrated, however, by the implantation of pellets that many of the other steroid hormones (cf. 4) are metabolized by the liver. Therefore, a similar study was undertaken with progesterone.

METHODS. Adult female rabbits which had been isolated for at least four weeks were used in all of the experiments. At the time of implantation of the pellets a piece of the right uterine horn was removed for histological study and the ovaries were carefully examined for corpora lutea. In a few instances the ovaries were removed but there was no difference in response between these animals and non-ovariectomized animals of the respective groups. On the seventh day after implantation of the pellets, the animals were killed and a piece of the left uterine horn and the ovaries were removed for histological study. The pellets were removed, washed in water, dried in a desiccator and reweighed on a torsion balance. The degree of progestational activity was evaluated by the Corner-Allen scale (5, 6).

Pellets. The progesterone² was prepared in pellets of two sizes: (a) $50 \pm$ mgm. concave discs, 6 mm. in diameter, and (b) $25 \pm$ mgm. cylinders, 3 mm. in diameter by 2 mm. in length. The latter were used only in the multiple mesentery experiments while the former were used in all the other experiments.

Sites of implantation of the pellets. The pellets were implanted under the skin in the inguinal region, in the right rectus abdominis muscle, in a fold of the mesentery (drainage to the liver) and in the right kidney.

RESULTS. The pellets of progesterone implanted subcutaneously and intramuscularly produced very similar positive progestational responses (table 1). The material in the kidney, however, was slightly less effective than the pellets implanted subcutaneously or intramuscularly. The effectiveness of the kidney implants is even less when calculated on the basis of per milligram of progesterone absorbed. The hormone implanted in the mesentery (drainage to the liver) is ineffective even in the multiple experiments in which an average of 20.2 mgm. of progesterone was absorbed; or about seven times the amounts absorbed from the pellets implanted subcutaneously or intramuscularly.

The rate of absorption of the progesterone varies with the site of implantation of the hormone. The kidney and mesentery have a much greater solvent effect on the hormone than the muscle and subcutaneous tissue (table 1).

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² The progesterone (Lutoeylin) was provided by the Ciba Pharmaceutical Products, Inc.

TABLE 1
The effect of various tissues on the activity of progesterone

SITE OF IMPLANTATION	NO. OF RABBITS	ENDOMETRIAL* REACTION	PROGESTERONE ABSORBED	RELATIVE† EFFICACY
			<i>mgm.</i>	
Subcutaneous.....	6	3.2+ (3+-4+)	3.0	1.1
Muscle.....	8	3.2+ (3+-4+)	2.6	1.2
Kidney.....	6	2.8+ (2+-3+)	5.1	0.5
Mesentery.....	6	0	4.7	0.0
Mesentery‡.....	4	0	20.2	0.0

* Corner Allen scale.

† Relative efficacy equals endometrial response divided by milligrams progesterone absorbed.

‡ Duration of experiment 14 days with 4 pellets ($25 \pm$ mgm. each) implanted in each rabbit.

DISCUSSION. The ability of the liver to inactivate progesterone even when as much as 20.2 mgm. was absorbed designates this organ as an important site for the metabolism of progesterone.

The much lower relative efficacy of the progesterone absorbed from the kidney implants suggests that this organ, too, may be effective in metabolising progesterone. More precise evidence to establish this fact could be obtained if pellets of progesterone were implanted such that direct renal drainage would occur as in the case of the mesentery-liver relationship. Unfortunately, the kidney does not lend itself to such an experiment.

The inactivation of progesterone by the liver and possibly the kidney provides an explanation for the greater responsiveness of the uterine endometrium to the direct application of this hormone; only two per cent of the amount by subcutaneous injection is required (7).

SUMMARY

In order to determine the effect of various tissues on the activity of progesterone, pellets of this compound were implanted in the mesentery (drainage to the liver), in the kidney, in the muscle and under the skin of adult, isolated female rabbits. The liver proved to be the chief site of inactivation of the hormone. The kidney also metabolised the compound, but to a much less degree. The pellets under the skin and in the muscle produced marked endometrial proliferation of approximately equal amount.

REFERENCES

- (1) VENNING, E. H. AND J. S. L. BROWNE. *Endocrinology* 21: 711, 1937.
- (2) ZONDEK, B. *Nature* 143: 282, 1939.
- (3) HEARD, R. D. H., W. S. BOULD AND M. M. HOFFMAN. *J. Biol. Chem.* 141: 709, 1941.
- (4) BISKIND, M. S. AND G. S. BISKIND. *Endocrinology* 32: 97, 1943.
- (5) CORNER, G. W. AND W. M. ALLEN. *This Journal* 88: 326, 1929.
- (6) ALLEN, W. M. *This Journal* 92: 174, 612, 1930.
- (7) MCGINTY, D., L. ANDERSON AND N. MCCULLOUGH. *Endocrinology* 24: 829, 1929.

VISUAL THRESHOLDS AS AN INDEX OF PHYSIOLOGICAL IMBALANCE DURING ANOXIA

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Constancy of the internal environment of an organism has been recognized as necessary for stable functioning, since Claude Bernard created the concept of the "milieu interieur." Independence of the organism from the limitations of the external environment exists only to the degree that the automatic physiological processes are capable of maintaining constancy of factors in the internal environment, such as oxygen tension, water, salts, sugar, hydrogen ion concentration and body temperature. The organism shows a remarkable capacity to prevent alterations and disturbances in these internal constants within the ordinary range of variations of the external environment, and is able to function normally. When certain limits are exceeded, an interruption of normal function results. The demarcation of these limits is not sharp. The deterioration in function, prior to complete breakdown, is gradual in onset, progressive and often insidious in nature. The central nervous system, particularly, is affected. These early stages of derangement, which are of the most interest to those concerned with the practical implications of lowered mental and physical efficiency, may be characterized as a state of physiological imbalance.

The precision of quantitative studies concerning physiological imbalance is limited, in part, by the relationship between the following factors: *a*, the sensitivity of the reaction being studied; *b*, the errors involved in making the measurements; and *c*, the temporary masking of the impairment by exerting additional effort. For example, the effects of oxygen deprivation were originally believed to exist only at much higher altitudes than is now known to be true. This misconception existed either because the functions studied were relatively stable during anoxia or because the methods of measurement lacked sufficient precision and enabled the subject to conceal the impairment. Tests of certain visual functions, particularly light sensitivity, largely avoid these difficulties. Changes of considerable magnitude are manifested by the visual mechanism, when its oxidative processes are disturbed. The physical measurements involved in these visual tests can be made very accurately, in comparison with measurements of other psycho-physiological functions. Moreover, the control of such experiments is simplified by the fact that the subject is not aware of changes in his own visual sensitivity. He does not know what changes in the physical intensity of the stimulus are necessary in order for him to see it, since at his threshold the stimulus always has the same appearance.

¹ This research has been aided by a grant from the Lockheed Aircraft Corporation. Acknowledgment also is made to the Linde Air Products Company for the supplies of nitrogen and oxygen used in these experiments.

Visual measurements during anoxia, or other stresses, have significance in addition to their value as an index of physiological imbalance. Their direct and practical application is obvious in the services and in industry, especially in aviation. Secondly, this information is of value in relation to the physiology of vision.

The effects of anoxia on vision have been reviewed by McFarland, Evans and Halperin. A number of investigators have described marked changes in dark adaptation, or absolute thresholds, during oxygen deprivation. These measurements consist of determining the least intensity of light, visible against a totally dark background. This may be considered a special case of intensity discrimination, in which one of the contrasted intensities is zero. No studies of the effects of anoxia on intensity discrimination as a function of light intensity have appeared in the literature.² The latter measurements involve the determination of light thresholds against backgrounds with various intensities of illumination. Since in most practical situations the background, against which an object is viewed, is not completely dark but is more or less illuminated, these measurements are more directly applicable to aviation. Furthermore, they were found in the present study to provide a more convenient and reliable index of the effects of anoxia.

In the present investigation foveal intensity discrimination was studied as a function of light intensity and of the degree of anoxia produced by lowering the partial pressure of oxygen, as described below.

METHODS. *Low Oxygen Apparatus. Chamber.* The first series of experiments was performed in a low-oxygen chamber (8 x 7 x 7 feet) designed by Barach. The oxygen tension was lowered by diluting the air with nitrogen, and maintained at the desired level by a continuous inflow of nitrogen compensating for the small amount of oxygen from the nasal catheter worn by the experimenter. Samples of the chamber air were obtained at the beginning and near the end of exposure to the reduced oxygen tension. The total atmospheric pressure was not altered. The temperature and humidity were maintained at a comfortable level by means of an air-conditioning unit. Fans within the chamber mixed the air thoroughly.

Mask. In the second series, experiments were performed at stages of oxygen deprivation which were progressively more severe. The oxygen tension of the air in the chamber as a whole was not altered. Instead, various mixtures of oxygen and nitrogen were inhaled by the subject from a rubber mask³ which covered the mouth and nose and fitted snugly against the face. Cylinders of the desired gas mixtures were prepared and their composition verified by analysis

² Crude measurements of the effects of 8 to 10 per cent oxygen on visual intensity discrimination were made by Schubert (Pflüger's Arch. 231: 1, 1932) and by Gellhorn (This Journal 115: 679, 1936), with the use of Masson discs. When these discs are rotated, a series of rings of different shades of gray are seen. The number of rings that can be seen is a measure of intensity discrimination. A considerable impairment of this function was reported.

³ Made by the Foregger Co., Inc., New York City; catalogue no. 22-4, with special chimney for expiratory valve.

of samples. All the cylinders were connected to a common manifold, making it possible to change from one to another with ease and without letting the subject know the change had taken place. The gases entered a large rubber bag from which they passed to the mask through a flutter valve during inspiration; this valve closed during expiration, and the expired gases passed into the room through a second flutter valve attached to the mask. When desired, instead of the prepared gas mixture, the air in the chamber could be admitted to the mask.

Analytical Methods. The gas samples were analyzed for oxygen and carbon dioxide with a standard Haldane apparatus. The blood samples were analyzed in duplicate for oxygen content and capacity by the micromethod of Roughton and Scholander. Heparinized blood samples were obtained anaerobically and without stasis from the dorsal veins of the hand after "arterialization" by immersion of the hand and wrist in water at a temperature of 45 to 47° C. for ten minutes (see Goldschmidt and Light).

Visual Apparatus. The apparatus employed for the visual measurement in the present study was a duplicate of the "discriminometer" described in detail by Crozier and Holway (1939). This instrument permits the precise and independent control of the intensity, the wave length composition, the area, the retinal location, and the duration of exposure of three stimuli on either one, or both, of the subject's retinas.

A simplified schematic plan of the discriminometer and the optical system used in this experiment are shown in figure 1 A. The apparatus consists essentially of the following components: *a*, three sets of quadrilateral slit edges *SI* define the stimuli and permit the independent adjustment of their length, width, and retinal location; *b*, the source *S* and the collimating systems *L*₁ and *L*₂ illuminate these apertures uniformly; *c*, a second triple optical system produces enlarged images of these apertures, arranged or superimposed in any desired pattern. The observer looks into the eyepieces of an inclined binocular microscope head *MH* which, together with lenses *L*₄ and *L*₃, is focussed on the slit edges.

The light source *S* is a special wide-ribbon tungsten filament projection lamp, operated from 110 volt lines through a variac and transformers. It is controlled for constant current with the aid of a Weston, model 155, ammeter. The intensity of each of the three beams is controlled independently by means of neutral tint Wratten filters *F* and balanced wedges *W*. The exposure time of any beam can be controlled by means of a camera shutter *C* placed in its path.

Calibration of the intensity of retinal illumination¹ produced by one of the beams was performed by binocular comparison with a Macbeth illuminometer, provided with an accurately measured artificial pupil. The other beams were calibrated by monocular comparison with the first. The filters and wedges were calibrated photometrically, by monocular comparison in the discriminometer with a series of filters of known optical density.

¹ Intensity of retinal illumination is expressed in photons (millilamberts $\times \frac{10}{\pi} \times$ pupil area in square millimeters). All values are given in milliphotons in order to avoid negative logarithms in expressing very low intensities.

The subject was protected from stray light by means of a cubicle built about the head of the discriminometer, and draped with heavy black velvet curtains. A fan provided ventilation within the cubicle. A Bausch and Lomb combination chin-rest and head-support was employed.

Conditions of Operation. In the present study the three beams of the discriminometer were adjusted as follows: *a.* The slit edges limiting one beam were opened fully, so that this beam filled the entire field seen in the ocular. This provided a uniformly illuminated background, circular in shape, subtending a

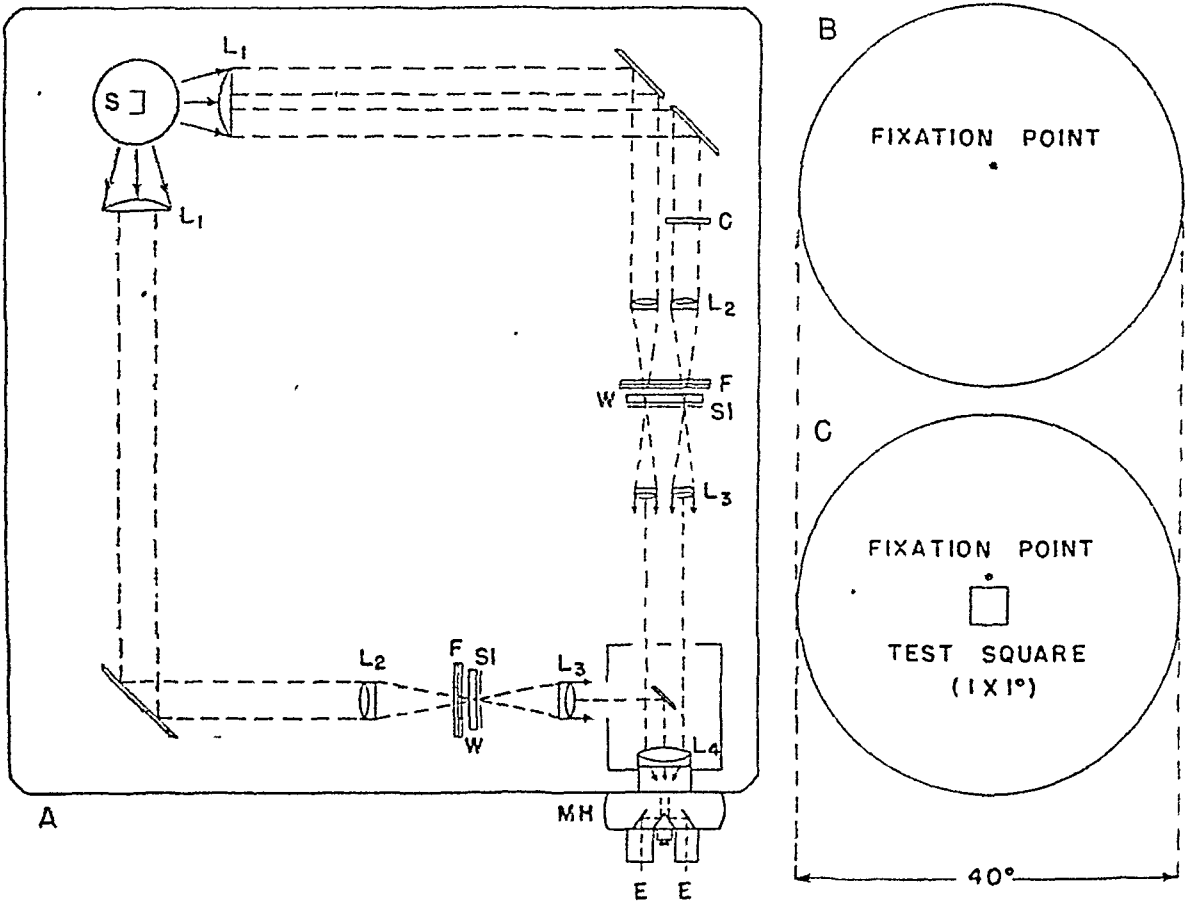


Fig. 1. A. Schematic diagram of the visual discriminometer. See text for description. B. Appearance of stimulus between exposures of the test square. C. Appearance of stimulus during exposure of test square.

visual angle of 40° , for the intensity discrimination measurements. It was also used as a light-adapting field preliminary to dark-adaptation determinations. The intensity of retinal illumination produced by this beam is referred to as *I*. *b.* The second beam was adjusted to furnish a small fixation point near the center of the field. It was provided with a deep red filter (Wratten no. 70). The wedge controlling the intensity of the fixation point could be adjusted by the subject, who maintained it at a "just visible level" throughout the experiment. These two beams were visible continuously during intensity discrimination measurements, as shown in figure 1 B. During measurements of dark adaptation,

the beam illuminating the field was interrupted, so that only the fixation point was visible. *c.* The third beam was adjusted to furnish a $1 \times 1^\circ$ square test stimulus, located just below the fixation point, as shown in figure 1C. Its exposure time was limited by a camera shutter adjusted to $\frac{1}{10}$ second. The intensity of retinal illumination produced by this test stimulus is denoted by ΔI . When superimposed on the field of intensity I , this stimulus results in a total intensity of $I + \Delta I$ in the test area.

All observations were made with the right eye; only the right ocular of the microscope head was used. The maximal diameter of the light beam at the eye ring of the ocular, less than 2 millimeters, was smaller than the natural pupil and thus served as an artificial pupil.

EXPERIMENTAL PROCEDURE. The procedure for dark-adaptation determinations was as follows. After preliminary exposure to a brightly illuminated field [$\log_{10} (I, \text{ in milliphotons}) = 8.072$] for 3 minutes, thresholds were determined every $\frac{1}{2}$ to 1 minute for approximately 15 minutes under the conditions described above. All threshold measurements were made in the ascending direction by flashing the test square at increasing intensities and at intervals of a few seconds until a positive response was obtained. Each complete determination required about 15 seconds.

Intensity discrimination measurements were then made in the order of increasing field intensities (I). Before measurements were taken at each field intensity, the subject was adapted to the field for a period of 3 to 5 minutes. The intensity of the test square was increased in steps of about $0.03 \log_{10}$ units until its outlines were just visible. At each field intensity ten measurements were obtained in 3 to 5 minutes.

Two types of experiments were performed:

A. The effect of a constant low oxygen tension on differential and absolute visual thresholds. These experiments were performed in the low-oxygen chamber. A dark adaptation curve was determined, followed by measurements of the intensity discrimination thresholds at 5 or 6 field intensities in normal air (21 per cent oxygen). Nitrogen was then admitted to the sealed chamber at such a rate as to lower the oxygen content of the air to about 11 per cent in 25 minutes. After 10 minutes of exposure to the reduced oxygen tension, the above procedure was repeated. The total duration of exposure to the low oxygen tension was about 75 minutes. In order to determine the reversibility of the changes produced by anoxia, the subject was then given a nasal catheter through which 100 per cent oxygen was delivered at a rate of 8 liters per minute. Immediately thereafter light adaptation to the bright field was repeated for 3 minutes. Dark-adaptation thresholds were determined 10 minutes later for a period of about 5 minutes. This was followed by intensity discrimination measurements at a selected low field intensity.

Control experiments were performed in the same manner as outlined for the low-oxygen experiments. All details of the low-oxygen experiments were simulated precisely so that the subject would not be aware of any differences in procedure. For example, a small amount of nitrogen was admitted to the chamber

in order to simulate the noises produced in the low-oxygen tests. However, the door of the chamber remained open and adequate ventilation was provided so that the oxygen content of the air was not altered. The subjects were not aware that these were control experiments. Some reported mild symptoms, suggestive of those which they experienced during the low-oxygen experiments.

B. *The relation between the severity of oxygen deprivation and the magnitude of its effect on differential brightness sensitivity.* In these experiments the mask was used to administer gas mixtures containing the desired percentages of oxygen. It was worn throughout each experiment. The subject was not aware of the time when he was first exposed to a low oxygen tension, or of any subsequent changes in the composition of his inspired air. The initial observations were made in normal air (21 per cent oxygen) until constant measurements were obtained. They were preceded by adaptation to a bright field ($\log_{10} I = 8.072$) for 3 minutes, and dark adaptation for 10 minutes for the sake of uniformity. All differential threshold measurements were made at a very dim field intensity ($\log_{10} I = 2.360$). Ten measurements were taken in about 3 minutes, and the subject was then allowed to alter his posture and rest for 2 minutes. This was repeated every 5 minutes. After several groups of measurements in normal air, similar determinations were made at each of five progressively more severe levels of oxygen deprivation. The duration of exposure to each level was 20 to 30 minutes. "Arterialized" venous blood samples were obtained at each level. Finally, 100 per cent oxygen was administered and similar measurements were taken during recovery.

RESULTS. The data obtained in the two types of experiments are presented below:

A. *The Effect of a Constant Low Oxygen Tension on Differential and Absolute Visual Thresholds.* Observations were made by nine trained subjects who had no physical or ocular abnormalities other than mild refractive errors. All were male students, ranging in age from 18 to 26 years. From one to three experiments were made on each subject. In addition, a control experiment was carried out on each of four of these subjects.

Low-oxygen experiments. Data for two groups of subjects (I and II) are presented separately, because of differences in the light intensities employed. Essentially identical results were obtained for both groups.

The logarithms of the differential thresholds ($\log_{10} \Delta I$) at each field intensity are presented in tables 1 and 2. Each datum represents a mean of 10 to 30 measurements, as indicated by the value of n . Group means are also presented in these tables and are shown graphically in figures 2 B and D.

Oxygen deprivation causes a decrease in differential brightness sensitivity, as demonstrated by an increase in the logarithm of the differential threshold. This increase is greatest at the lowest field intensities and becomes progressively smaller as the field intensity becomes brighter. At the highest field intensity employed in the present study, it is almost zero. The mean changes in the differential thresholds for each group are shown in table 3.

In order to demonstrate the reversibility of the effects of anoxia on differential

TABLE 1
Intensity discrimination data for group I
(See fig. 2)

CONDITIONS	LOG ₁₀ I	LOG ₁₀ Δ I (MILLIPHOTONS)									
		Subject								Group average n = 70	
		Mo n = 20		Hr n = 20		Go n = 10		Ws n = 20			
		Mean	s	Mean	s	Mean	s	Mean	s	Mean	s
Normal air (20.93% O ₂)	milli- photons										
	3.175	3.583	0.032	3.414	0.032	2.867	0.038	2.895	0.044	3.190	0.037
	3.682	3.758	0.029	3.601	0.029	3.032	0.035	3.075	0.038	3.366	0.034
	4.621	4.248	0.024	4.136	0.030	3.600	0.043	3.728	0.030	3.928	0.033
	6.145	5.837	0.025	5.378	0.033	4.858	0.050	5.036	0.033	5.277	0.036
	7.234	6.897	0.036	6.489	0.029	5.941	0.049	6.111	0.033	6.359	0.037
Low oxygen (mean % O ₂ = 10.81; mean % CO ₂ = 0.31)	3.175	3.895	0.039	3.626	0.034	3.269	0.025	3.087	0.050	3.469	0.041
	3.682	3.964	0.039	3.747	0.036	3.386	0.031	3.174	0.036	3.568	0.038
	4.621	4.419	0.044	4.214	0.024	3.832	0.033	3.750	0.046	4.054	0.041
	6.145	5.969	0.032	5.424	0.025	4.907	0.052	5.072	0.028	5.343	0.035
	7.234	6.904	0.029	6.504	0.048	5.924	0.041	6.117	0.044	6.362	0.042
100% O ₂	3.175	3.563	0.030	3.327	0.035	2.932	0.036	2.824	0.033	3.161	0.036

TABLE 2
Intensity discrimination data for group II
(See fig. 2)

CONDITIONS	LOG ₁₀ I	LOG ₁₀ Δ I (MILLIPHOTONS)									
		Subject								Group average n = 120	
		Ha n = 30		Al n = 30		Ph n = 20		Wi n = 20		Ni n = 20	
		Mean	s	Mean	s	Mean	s	Mean	s	Mean	s
	milli- photons										
Normal air (20.93% O ₂)	2.259	3.327	0.040	2.718	0.035	3.201	0.048	3.225	0.042	2.436	0.042
	2.598	3.466	0.033	2.714	0.030	3.221	0.029	3.312	0.030	2.531	0.017
	3.175	3.516	0.031	2.858	0.031	3.291	0.034	3.415	0.036	2.676	0.013
	4.621	4.274	0.034	3.706	0.041	3.859	0.032	4.109	0.031	3.667	0.035
	6.145	5.653	0.025	4.978	0.039	5.083	0.035	5.518	0.032	4.912	0.020
	7.234	6.721	0.027	6.169	0.032	6.134	0.034	6.680	0.029	5.984	0.048
Low oxygen (mean% O ₂ = 10.80; mean% CO ₂ = 0.35)	2.259	3.651	0.033	2.986	0.035	3.527	0.041	3.581	0.039	2.685	0.035
	2.598	3.737	0.036	2.925	0.013	3.536	0.039	3.579	0.031	2.715	0.051
	3.175	3.797	0.030	3.026	0.030	3.603	0.012	3.615	0.022	2.821	0.059
	4.621	4.401	0.032	3.706	0.036	4.001	0.037	4.235	0.025	3.626	0.037
	6.145	5.740	0.025	5.031	0.031	5.179	0.035	5.527	0.022	4.971	0.042
	7.234	6.672	0.030	6.208	0.027	6.160	0.026	6.682	0.027	5.938	0.030
100% O ₂	2.598	3.346	0.032	2.660	0.018	3.184	0.031	3.246	0.020	2.421	0.010

brightness sensitivity, oxygen was administered to the subjects at the end of each experiment. Measurements were repeated at a selected low field intensity at which anoxia had caused a marked elevation of the threshold. These measurements were made about 20 minutes after the administration of oxygen was begun. At this time, the thresholds were found to have dropped to their original values or even slightly lower. The differences between the final and original

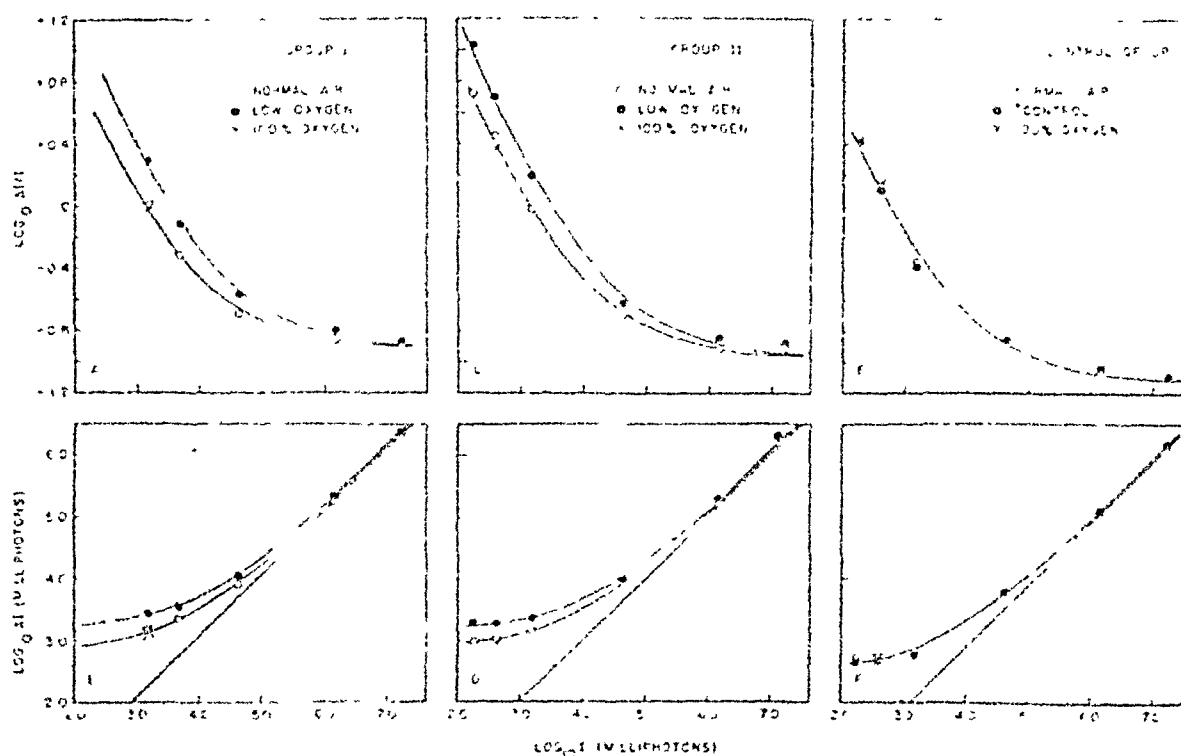


Fig. 2. The effect of oxygen deprivation on foveal intensity discrimination. The curves are theoretical, derived from Hecht's equation (see text). In A and C, the data are plotted as the Weber fraction $\Delta I/I$ against the intensity of illumination of the field. The theoretical curve was first fitted to the open circles and then translated horizontally until it fitted the solid ones. In B and D, the same data are plotted directly as the differential threshold. The straight diagonal line is the asymptote of the curves and has a slope of 1. The theoretical curve was first fitted to the open circles and then translated upward and to the right along the asymptote until it fitted the solid ones. The points indicated by the symbol X were obtained after recovery from anoxia by inhaling oxygen. (See tables 1 and 2.)

E and F represent control experiments in which reduction of oxygen tension was simulated but did not actually occur. No significant changes occurred. (See table 5.)

values of the thresholds were not significant for group I, but were statistically reliable in group II (table 3). Other experiments have not shown an improvement of visual sensitivity on administration of oxygen after breathing normal air without intervening anoxia (control data).

The variability of measurements for single experiments is represented by $s = \sqrt{\Sigma d^2/(n-1)}$, the standard deviation (i.e., the square root of the variance). The measure of variability of combined data from several experiments is given

by the square root of the pooled variance; the above formula becomes $s = \sqrt{[\Sigma d_1^2 + \Sigma d_2^2 \dots + \Sigma d_k^2]/[(n_1 - 1) + (n_2 - 1) \dots + (n_k - 1)]}$ for a series of k experiments, where Σd_k^2 is the sum of the squares of the deviations of the measurements from their mean in experiment k , and n_k is the number of measurements in that experiment (see Snedecor, p. 68). In the latter case s closely approximates a mean standard deviation.

The use of s , which is a measure of the average variability of the means for the individual experiments, is preferable to the standard deviation of the mean for the combined data. The latter would give a measure of the scatter of the means

TABLE 3

Statistical analysis of changes in visual thresholds in low-oxygen experiments

LOG ₁₀ I	GROUP I					GROUP II				
	Mean change	Standard deviation	Standard error	Critical ratio	P*	Mean change	Standard deviation	Standard error	Critical ratio	P*
Low oxygen compared with normal air										
<i>milli-photons</i>	<i>log₁₀ units</i>	<i>log₁₀ units</i>	<i>log₁₀ units</i>			<i>log₁₀ units</i>	<i>log₁₀ units</i>	<i>log₁₀ units</i>		
2.259						0.306	0.046	0.020	15.00	<0.01
2.598						0.250	0.052	0.023	10.73	<0.01
3.175	0.280	0.097	0.049	5.76	<0.01	0.223	0.073	0.033	6.82	<0.01
3.682	0.201	0.111	0.055	3.63	0.04					
4.621	0.126	0.094	0.047	2.69	0.08	0.071	0.085	0.038	1.87	0.14
6.145	0.066	0.044	0.022	2.97	0.06	0.061	0.034	0.015	3.99	0.02
7.234	0.003	0.014	0.007	0.41	0.71	0.036	0.034	0.015	0.39	0.72
100% oxygen compared with low oxygen										
2.598						-0.326	0.050	0.022	-14.63	<0.01
3.175	-0.308	0.034	0.017	-17.91	<0.01					
100% oxygen compared with normal air										
2.598						-0.077	0.035	0.016	-4.87	<0.01
3.175	-0.028	0.068	0.034	-0.82	0.47					

* P is the probability that a change greater than zero might be due to chance.

from different experiments due to such factors as changes in criteria, day-to-day variations in threshold sensitivity, and individual differences. Since these factors are controlled in any one experiment, they are extraneous in relation to the change caused by anoxia in a given experiment, or the average of such changes.

The standard deviations (s) of $\log_{10} \Delta I$, as computed above, are of the same order of magnitude at all intensities and show no consistent relationship to intensity. Thus, on an arithmetic scale, the variability is at all times approximately proportional to the threshold, as has been pointed out by Crozier and Holway (1937).

Although the values of s in tables 1 and 2 show a tendency for the variability of

the logarithm of the threshold (i.e., the percentage variability of the threshold itself) to increase during oxygen deprivation, the changes are small and inconsistent. The statistical reliability of these changes in variability was computed according to the method given by Garrett (p. 223) for each group at each intensity. Except for the measurements at $\log_{10} I = 2.598$ for group II, none of the changes was significant, the critical ratios ranging from -1.62 to $+1.71$. Furthermore, the difference between the pooled s for all the subjects at all intensities in normal air (0.035), as compared with low oxygen (0.037), was only $0.002 \log_{10}$ units, with a critical ratio of 1.82, indicating a lack of statistical reliability.

An increase in variability during oxygen deprivation has been found in a number of psychological and psycho-physiological tests, such as simple reaction time, choice reaction, pursuit meter, code test, memory test, and digit symbol test (McFarland). Presumably this reflects an increase in the biological variability of the organism. The lack of a significant increase in threshold variability in the present experiments is consistent with Hecht, Shlaer and Pirenne's assertions that "At the threshold where only a few quanta of energy are involved, it is the stimulus which is variable, and the very nature of this physical variability determines the variation encountered between response and stimulus. Moreover, even when biological variation is introduced, it is the physical variation which essentially dominates the relationship." Obviously the exposure of a subject to low oxygen tension cannot affect the physical variability of the light stimulus.

The statistical reliability of the changes in the differential thresholds was determined by Fisher's method for the significance of a unique sample.⁵ The results of the statistical analysis, shown in table 3, are closely similar for the two groups of subjects. At the lowest field intensities, the changes have a very high statistical significance as indicated by the high critical ratios and the extremely low values of P . The statistical significance of the mean changes decreases, on the whole, as the field intensity becomes greater. At the highest intensity, the change in $\log_{10} \Delta I$ is not significant statistically for either group. The decrease in the differential threshold during recovery with 100 per cent oxygen is highly reliable for both groups.

The mean results for each group of subjects are shown graphically in figure 2 A-D. Each set of data is represented: a , in terms of the traditional Weber fraction $\Delta I/I$, and b , directly as the differential threshold ΔI . In every case, the curves which were fitted to the points are those derived from Hecht's equation (see Discussion), based on the photochemical hypothesis. The agreement

⁵ The standard deviation of the change at each intensity, for each group of subjects, was first obtained by $s = \sqrt{\sum d^2 / (n - 1)}$, where d is the deviation of each change from the mean for the group, and n is the number of individuals comprising the group. The standard error of the mean change was then computed according to the formula $s_m = s/\sqrt{n}$. The critical ratio t is the ratio of the mean change to its standard error. The higher this ratio, the greater is the statistical reliability of the change. The corresponding value of P obtained from a "table of t " (Fisher, p. 167) denotes the probability that the change might be due to chance. Changes having a P of 0.05 or less are usually considered to be statistically significant, and values of 0.01 or less are considered to be highly significant.

of our observations with that equation is shown by the close fit of the curves to the data. The points representing the measurements at the highest field intensity fall above the theoretical curves. This may be due to a decrease in the critical duration to a value shorter than the stimulus exposure time at that intensity. Graham and Kemp, as well as Keller, have shown that as light intensity increases, the critical duration decreases. When the latter becomes shorter than the stimulus exposure time, not all of the stimulus is effective. Hence a greater stimulus intensity is needed and the points in the graph are elevated. In the study of Hecht, Peskin and Patt, the exposure time employed (0.04 sec.) was so short that it never exceeded the critical duration, and there was no rise in $\Delta I/I$ at the highest intensities.

The form of the relationship between either $\log_{10} \Delta I/I$, or $\log_{10} \Delta I$, and $\log_{10} I$ is not altered by oxygen deprivation; only its position on the co-ordinate axes is affected. When the data are plotted in terms of $\Delta I/I$ (fig. 2 A and C), a translation takes place only on the horizontal (I) axis; there is no vertical translation. The low-oxygen curves in these figures were drawn by shifting the normal-air curves to the right. Thus, the minimum value reached by $\Delta I/I$ at the highest intensity is not altered. When the data are plotted in terms of ΔI (fig. 2 B and D), the curves are translated on both axes. The magnitude of the shift with respect to each axis is equal to that of the $\Delta I/I$ curve on the horizontal axis. The result is a translation of the ΔI curve upward and to the right at an angle of 45° , along its asymptote (diagonal straight line in fig. 2 B and D), which has a slope of 1.

The magnitude of the shift of a curve expresses in a single number the magnitude of the effect of anoxia on foveal intensity discrimination. Thus, it is a more convenient measure than the change in differential threshold, which is a function of the field intensity. The data for each subject were plotted in a manner similar to figure 2 A and C, and the amount of translation of each curve during anoxia was determined graphically. These results are given in table 4. The statistical significance of the mean shift for the entire group of nine subjects is very high, with a critical ratio of 11.83.

Dark-adaptation curves were determined in order to compare the effect of anoxia on this function with that on visual intensity discrimination as expressed by the shift of the curve. Representative data, obtained during a single experimental session, are shown in figure 3 A. The absence of a discontinuity in the slope of the curve is interpreted as indicating that only cone vision is represented. This type of curve is obtained by the use of a small test object, foveally fixated. If measurements under these conditions are made over a longer time, a second dip of much smaller magnitude, indicative of some rod activity, may appear between the fifteenth and the twentieth minutes in the dark.

The upward translation of the dark-adaptation curve on the intensity axis during oxygen deprivation (fig. 3 A) indicates a decrease in light sensitivity. This impairment was reversed by administration of oxygen. Since a number of studies dealing with the effects of anoxia on dark adaptation have already been published, only the magnitude of the changes due to anoxia will be presented

here. These were determined for each experiment in the following manner. The mean value of the absolute threshold ($\log_{10} \Delta I_0$) during the tenth to fifteenth.

TABLE 4

A comparison between the effect of anoxia on intensity discrimination and on dark adaptation

SUBJECT	SHIFT OF INTENSITY DISCRIMINATION CURVE	SHIFT OF DARK-ADAPTATION CURVE
	<i>log₁₀ units</i>	<i>log₁₀ units</i>
Mo.....	0.52	0.32
Hr.....	0.31	0.41
Go.....	0.50	0.53
Ws.....	0.30	0.28
Ha.....	0.35	0.36
Al.....	0.32	0.32
Ph.....	0.32	0.29
Wi.....	0.31	0.32
Ni.....	0.27	0.36
Mean.....	0.356	0.354
Standard deviation.....	0.090	0.077
Standard error.....	0.030	0.026
Critical ratio.....	11.83	13.62
<i>P</i> *.....	<0.01	<0.01

* *P* is the probability that a change greater than zero might be due to chance.

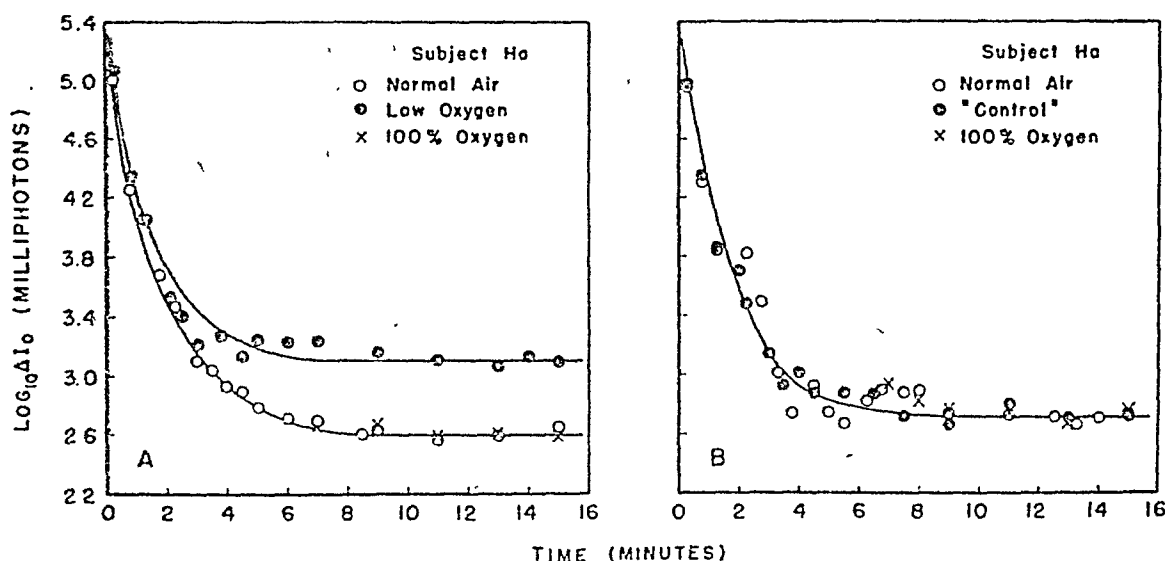


Fig. 3. A. The effect of anoxia on foveal dark adaptation. The thresholds ΔI_0 are plotted against time in the dark, after exposure for 3 minutes to an intensity of 118,000 photons. Anoxia causes a rise in the thresholds, reversible by oxygen. B. Control data, from an experiment in which the decrease in oxygen tension was simulated but was not actually brought about. All the points are fitted by a single curve.

minutes in the dark was determined graphically for each curve. The levels reached in normal air and in 100 per cent oxygen were averaged. The difference between this mean and the absolute threshold in low oxygen was then calculated.

Table 4 indicates the mean change for each subject. The statistical significance of the average of these changes for each group of subjects was computed by Fisher's method outlined above. It was found to be very high, the critical ratio being 13.62.

In general, the increase in the absolute threshold is slightly greater than the increase in the differential threshold, even at the lowest field intensity employed. The mean shift of the dark-adaptation curves ($0.354 \log_{10}$ unit) was, however, practically identical with the mean shift of the intensity discrimination curves ($0.356 \log_{10}$ units). The statistical significance of the difference between these

TABLE 5
Intensity discrimination data for control group
(See fig. 2)

CONDITIONS	LOG ₁₀ I	LOG ₁₀ Δ I (MILLIPHOTONS)									
		Subject								Group average n = 40	
		H _α n = 10		Al n = 10		W _i n = 10		Ph n = 10			
		Mean	s	Mean	s	Mean	s	Mean	s	Mean	s
Normal air	milli- photons										
	2.259	2.690	0.048	2.670	0.032	2.822	0.036	2.613	0.020	2.699	0.038
	2.598	2.711	0.041	2.659	0.042	2.932	0.032	2.643	0.030	2.736	0.035
	3.175	2.750	0.034	2.759	0.017	3.046	0.034	2.713	0.029	2.817	0.028
	4.621	3.717	0.038	3.641	0.033	3.816	0.058	3.792	0.020	3.741	0.039
	6.145	5.112	0.030	4.975	0.036	5.265	0.053	5.038	0.037	5.030	0.040
7.234	6.122	0.046	5.984*	0.036	6.243	0.020	6.062	0.025	6.103	0.034	
Normal air control	2.259	2.664	0.011	2.532	0.035	2.859	0.039	2.647	0.026	2.675	0.039
	2.598	2.669	0.038	2.527	0.045	2.931	0.052	2.665	0.033	2.698	0.048
	3.175	2.741	0.024	2.594	0.023	3.062	0.023	2.750	0.023	2.787	0.023
	4.621	3.741	0.035	3.629	0.030	3.927	0.037	3.780	0.028	3.769	0.033
	6.145	5.087	0.011	4.865	0.037	5.328	0.025	5.103	0.023	5.035	0.033
	7.234	6.148	0.026	5.981*	0.037	6.312	0.024	6.129	0.023	6.143	0.029
100% O ₂	2.598	2.669	0.025	2.581	0.012	3.048	0.023	2.623	0.030	2.730	0.031

* Extrapolated graphically.

means was negligible, the critical ratio being 0.03, and the corresponding value of P , 0.96. This relationship is discussed more fully below (cf. p. 315).

Control experiments. These were made to determine whether factors other than the change in oxygen tension might be responsible for the above effects. The differential thresholds of four subjects are given in table 5.

No consistent or appreciable changes in these thresholds or in their variability were observed during the pretended exposure to low oxygen tensions ("control"), or during subsequent administration of oxygen. It is evident in figure 2 E and F that all the data are fitted by a single curve. Furthermore, statistical analysis of the changes in $\log_{10} \Delta I$ during the control experiments (table 6) revealed that

none of these changes are significant. The results of the dark-adaptation measurements similarly showed no change to have occurred. Representative dark-adaptation data are presented in figure 3 B.

B. *The Relation Between the Severity of Oxygen Deprivation and the Magnitude of its Effect on Differential Brightness Sensitivity.* The second part of this investigation was designed to study the magnitude of changes in differential sensitivity as a function of oxygen tension. This problem was investigated by measuring the differential threshold at a selected low intensity of illumination because the effect of anoxia is greatest under this condition of light intensity.

Measurements of $\log_{10} \Delta I$ were made at a field intensity of about 230 milliphotons ($\log_{10} I = 2.360$). This is equivalent to a luminous intensity of about

TABLE 6
Statistical analysis of changes in visual thresholds in control experiments

$\log_{10} I$	MEAN CHANGE	STANDARD DEVIATION	STANDARD ERROR	CRITICAL RATIO	P^*
"Control" compared with normal air					
<i>milliphotons</i>	<i>log₁₀ units</i>	<i>log₁₀ units</i>	<i>log₁₀ units</i>		
2.259	-0.023	0.082	0.041	-0.57	0.61
2.598	-0.038	0.068	0.034	-1.12	0.35
3.175	-0.030	0.092	0.046	-0.65	0.56
4.621	+0.028	0.058	0.029	+0.97	0.40
6.145	+0.005	0.091	0.046	+0.11	0.92
7.234	+0.040	0.034	0.017	+2.35	0.10
100% oxygen compared with "control"					
2.598	+0.032	0.069	0.034	+0.94	0.42
100% oxygen compared with normal air					
2.598	-0.006	0.085	0.042	-0.14	0.90

* P is the probability that a change greater than zero might be due to chance.

0.002 foot candles viewed through the natural fully dilated pupil. Observations were made while breathing normal air and during administration of a series of five O_2-N_2 mixtures with a progressively decreasing oxygen concentration. The detailed results for one subject are given in figure 4. Each point represents the mean of ten observations. The distance from the center of each point to the ends of the vertical lines is equal to the standard deviation of $\log_{10} \Delta I$, thus representing $\pm s$. The standard errors are about one-third as great ($s_m = s/\sqrt{10}$). During a control experiment (fig. 4 A) the threshold remained at the same level for two hours. The low-oxygen experiment is represented by figure 4 B. The differences between the levels reached at each altitude and the initial levels of $\log_{10} \Delta I$, determined graphically, are plotted against the equivalent altitudes in figure 5 A, for two subjects. (The altitudes, which are equivalent to the various oxygen percentages, are according to data for the U. S.

standard atmosphere at a constant temperature of 15°C.) The curves have a sigmoid shape. The initial portion of each curve is similar to the parabolic curve given by McFarland and Evans in reporting dark-adaptation studies at

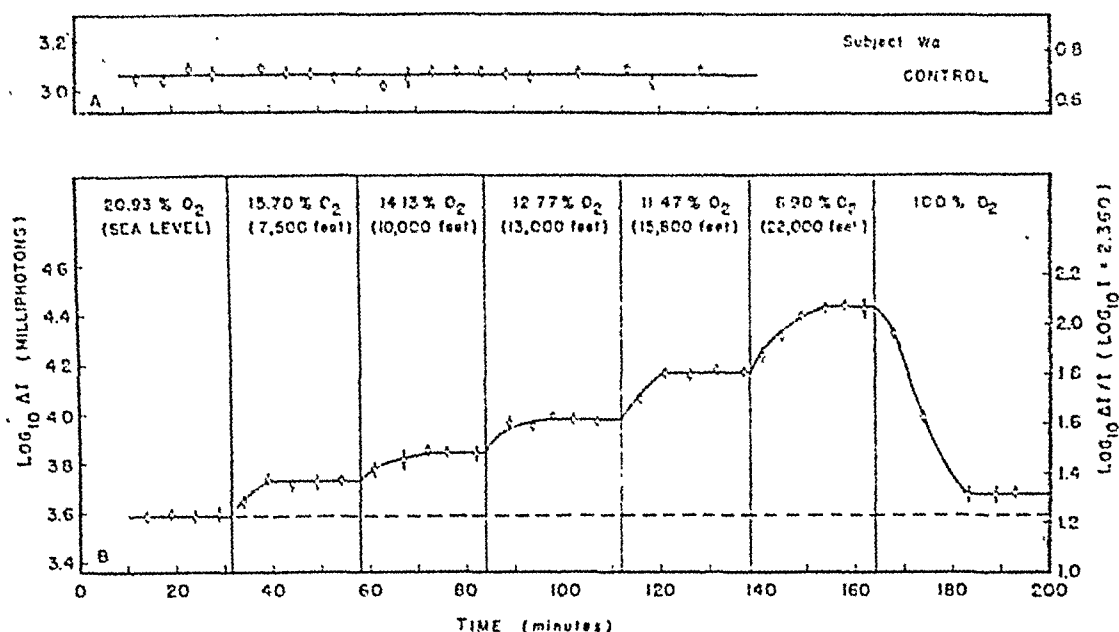


Fig. 4. The effect of progressive degrees of oxygen deprivation on foveal differential thresholds, at a constant field intensity. Each point represents the mean of ten observations. The distance from the center of each point to each end of the vertical line through it represents the standard deviation. In A the oxygen tension was not altered.

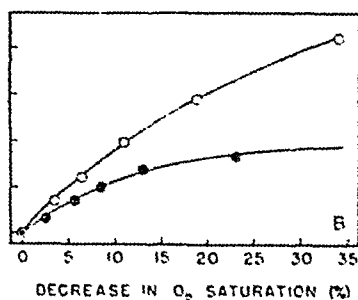
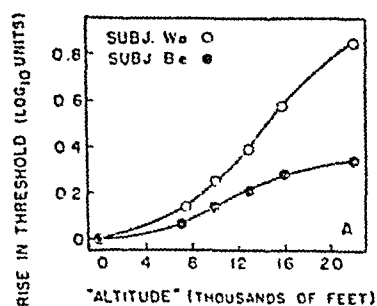


Fig. 5

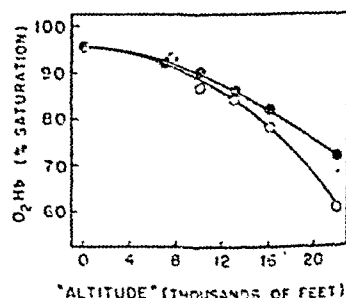


Fig. 6

Fig. 5. A. The relation between simulated altitude and the resultant rise in visual thresholds. B. The relation between the decrease in blood oxygen saturation, as a result of the simulated altitude (see fig. 6), and the associated rise in visual thresholds.

Fig. 6. Blood oxygen saturation in relation to simulated altitude. Open circles represent data for subject Wa and solid circles for subject Be.

simulated altitudes of up to 15,000 feet and to that given by Wald, Harper, Goodman and Krieger. The curvature of the first portion of each graph is closely similar to that of the graphs representing the "arterialized" venous oxygen saturation (fig. 6), if the latter are inverted. When the rise in threshold is plotted in relation to loss of oxygen saturation, therefore, the initial portion of

each curve shows an almost linear relationship (fig. 5 B). At lower oxygen tensions, the curve tends to become more horizontal.

DISCUSSION. A variety of mathematical descriptions of the relationship between differential sensitivity and light intensity have been proposed. None of these may be considered unique, since more than one such proposed description provides satisfactory agreement with the same sets of experimental observations. Furthermore, the fact that a given theoretical equation fits a set of observations does not constitute proof that the underlying theory is the correct one. Such a fit merely indicates the possibility that the theory may offer the correct explanation of the underlying mechanism. Nevertheless, such theoretical equations and curves are useful in describing the experimentally determined relationships.

The intensity discrimination measurements made in the present study have been fitted with curves derived from an equation proposed by Hecht in 1934. Several studies have shown that this equation provides a very close fit to experimental measurements of the intensity discrimination function for the human eye (Steinhardt; Hecht, Peskin and Patt; Graham and Kemp). This equation is based essentially on the following supposition. In order to discriminate between an intensity I and another just perceptibly brighter intensity $I + \Delta I$, the added intensity ΔI must be of such magnitude as to produce a constant increment in the photochemical decomposition in a given time. When the exposure to ΔI is constant, this is equivalent to saying that a constant initial photochemical change must be produced by ΔI , in order that its addition to I will be just perceptibly recognized. When this hypothesis is applied to the equilibrium (or "stationary state") equation for the kinetics of the decomposition and regeneration of a photosensitive substance, several equations may be derived depending on the orders of the chemical reactions. The one which describes the data for human cone vision, and which was used to fit the present data, is:

$$\frac{\Delta I}{I} = C \left[1 + \frac{1}{(KI)^{\frac{1}{2}}} \right]^2 \quad \text{or} \quad \Delta I = CI \left[1 + \frac{1}{(KI)^{\frac{1}{2}}} \right]^2$$

Hecht has pointed out that when these equations are plotted on a double logarithmic grid (i.e., $\log_{10} \frac{\Delta I}{I}$ against $\log_{10} I$), the form of the resulting curve is independent of the values of C and K . These constants merely define the position of the curve on the co-ordinate axes. Therefore, the theoretical curves may be plotted using arbitrary values of C and K , and then be superimposed directly on a double logarithmic plot of the experimental data.

The curve drawn in figure 7 A corresponds to the first form of the equation given above. As $\log_{10} I$ decreases, the curve approaches an asymptote which has a slope of -1 . As the light intensity increases indefinitely, the curve approaches another asymptote, which is horizontal. The point of intersection of these asymptotes may be used to define the position of the curve as a whole.

The location of this point is at $\log_{10} I = -\log_{10} K$ and $\log_{10} \frac{\Delta I}{I} = \log_{10} C$.

Thus C represents the limiting value of $\frac{\Delta I}{I}$ at high intensities and determines the position of the curve on the axis of ordinates. Similarly, the value of K determines the position of the curve on the axis of abscissas.

The data in figure 2 A and C indicate that oxygen deprivation results in a translation of the $\log_{10} \frac{\Delta I}{I}$ vs. $\log_{10} I$ curve to the right. Consequently, the constant K becomes smaller. Since this translation has no vertical component, the magnitude of the constant C remains unchanged. Thus the minimum value

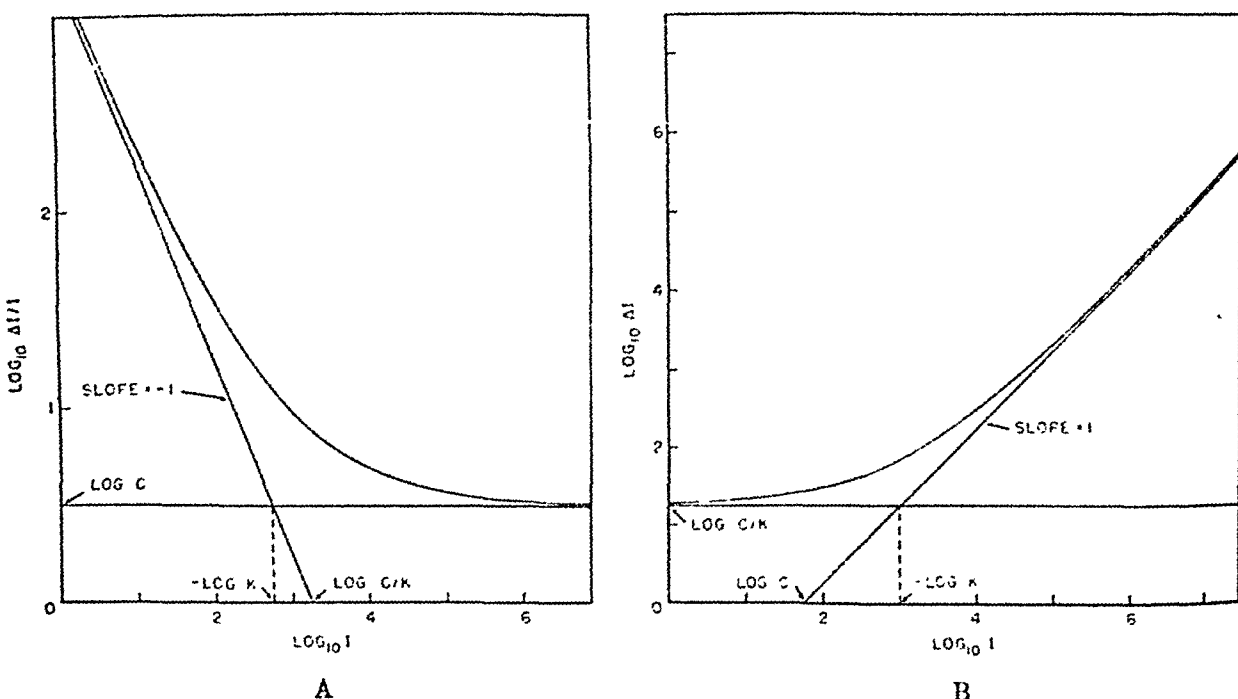


Fig. 7. Analysis of the theoretical curves, based on Hecht's equation.

of $\frac{\Delta I}{I}$ at high light intensities is not affected by anoxia. As the intensity decreases and the slope of the curve becomes greater, the change in $\log_{10} \frac{\Delta I}{I}$ becomes larger. The translation of this curve on the intensity axis during anoxia is similar, in direction and magnitude, to that of the curves relating visual acuity (McFarland and Halperin), or latency of after-images (McFarland, Hurvich and Halperin) to intensity. In all these cases, the effect of anoxia is comparable to placing an optical filter before the subject's eye, thus necessitating a greater light intensity to produce a given visual response.

The curve drawn in figure 7 B corresponds to the second form of the equation given above. As $\log_{10} I$ decreases, the curve approaches a horizontal asymptote. As $\log_{10} I$ increases indefinitely, the curve approaches another asymptote which has a slope of $+1$. The point of intersection of these asymptotes may likewise be used to define the position of the curve. This point is located at $\log_{10} I =$

$-\log_{10} K$, and $\log_{10} \Delta I = \log_{10} C/K$. As shown in figure 7 B, the value of C determines the position of the diagonal asymptote. The value of C/K determines the position of the curve on the ordinate and that of K its position on the abscissa. A given change in $\log_{10} K$ produces an equal change in $\log_{10} C/K$ (or $\log_{10} C - \log_{10} K$). Therefore, a decrease in K causes an equal displacement of the curve to the right and upward along the diagonal asymptote.

The points in figure 2 B and D were fitted with this theoretical curve in the following manner. First, the curve was superimposed and drawn on the points obtained in normal air; the diagonal asymptote was also drawn. The curve was then translated upward and to the right along the asymptote until it coincided with the low-oxygen data. The magnitude of this translation was such that both its horizontal and vertical components were equal to the amount of shift of the curves in figure 2 A and C on the horizontal axis. It is evident that this change is equivalent to a decrease in the value of the constant K . When the data are plotted in this manner, it is obvious that anoxia causes an increase in the differential threshold which is greatest at the lowest field intensity, and becomes progressively smaller as the field intensity is increased. For a given value of $\log_{10} I$, the change in $\log_{10} \Delta I$ is, of course, identical with the change in $\log_{10} \Delta I/I (= \log_{10} \Delta I - \log_{10} I)$.

The effect of anoxia on the several visual functions which have been studied thus far may be described as a translation of the curve along that axis which has the dimension of light intensity. Thus, in the case of the curves for visual acuity *vs.* $\log_{10} I$, or of latent period of after-images *vs.* $\log_{10} I$, there is a translation to the right on the intensity axis. In the case of dark-adaptation curves, where the thresholds ($\log_{10} \Delta I_0$) are plotted against time in the dark, the curve is translated upward on the axis of threshold intensities. When the intensity discrimination function is plotted as $\log_{10} \Delta I/I$ *vs.* $\log_{10} I$, only the latter variable has the dimension of intensity; $\Delta I/I$ is a ratio with no dimensions. Thus, the curve is displaced only to the right, on the $\log_{10} I$ axis. However, when $\log_{10} \Delta I$ is plotted against $\log_{10} I$, both variables have the dimensions of intensity. Accordingly, the curve shifts equally on both axes.

The absolute threshold (ΔI_0) for the dark-adapted eye may be considered as the limiting value of the differential threshold, when the field intensity is zero. The change in the absolute threshold during anoxia should, therefore, be explainable in terms of the translation of the intensity discrimination curve. This relationship may be described in the following way. As $\log_{10} I$ decreases, the slope of the $\Delta I/I$ curve (fig. 2 A and B) approaches an absolute value of 1. At a very low field intensity, therefore, the change in $\log_{10} \Delta I/I$ due to anoxia (as measured on the ordinate) approaches in magnitude the amount of horizontal displacement of the curve. For a given value of $\log_{10} I$ the change in $\log_{10} \Delta I/I$ is equal to the change in $\log_{10} \Delta I$. Therefore, the change in $\log_{10} \Delta I$ at an infinitely low field intensity (i.e., the change in the absolute threshold $\log_{10} \Delta I_0$) should be equal to the horizontal displacement of the intensity discrimination curve, both being expressed in \log_{10} units. The data in table 4, which show that the mean

upward translation of the dark-adaptation curve is equal to the mean shift of the intensity discrimination curve, bear out this expectation.

When a subject is exposed to a physiological stimulus such as oxygen deprivation, measurements of visual sensitivity plotted in relation to time (as in fig. 4) may be used to indicate the magnitude, and rate of alterations, of the induced changes. Although dark-adapted (or absolute) thresholds may be employed for this purpose, there are several reasons why the differential threshold at a very low field intensity offers advantages: *a.* The absolute threshold tends to keep diminishing even after comparatively long periods in the dark. This interferes with the interpretation of changes caused by the experimental conditions. On the other hand, a dimly illuminated field maintains the eye at a constant state of adaptation after the first few minutes of exposure. Control experiments showed no appreciable change during periods of over two hours. *b.* Less time is necessary to reach a stable state at the beginning of the experiment than in the case of absolute thresholds. *c.* The subject has less difficulty in fixating, when he sees a circular field as compared with total darkness. *d.* Entoptic phenomena are less disturbing. *e.* The results have more direct practical application to aviation since even night vision generally involves a background with very dim illumination rather than total darkness.

Changes in differential brightness sensitivity are significant not only as a measure of this specific function during exposure to a given physiological stimulus. In addition, they imply similar changes in other visual functions under the same conditions. The close relationship which exists between visual acuity, for example, and intensity discrimination has been noted repeatedly. Bartley points out that visual acuity is a special case of intensity discrimination in which the spatial relationships of an object are emphasized. This is due to the fact that under most circumstances objects can be seen against a given background only when there is a discriminable difference between the brightness of illumination of the object and that of its background. Hecht and Mintz, as well as Shlaer, Smith and Chase, have shown that the same mathematical relationship exists between $\Delta I/I$ and I as between the minimum resolvable angle (α) and I . When the experimental data for both these functions are plotted on a double logarithmic grid, they can be fitted by the same theoretical curve as used in the present study. Furthermore, Hecht and Mintz have computed, in terms of diffraction optics, the distribution of light in the retinal image of the finest line which was visible against a bright background. They found that the light falling on one row of cones differed in intensity from that incident on the adjacent rows by about 1 per cent. This value is approximately the same as that obtained by direct measurements of the differential threshold at similar light intensities. This finding is consistent with the view that differential sensitivity is the limiting factor for visual acuity in this case. Therefore, when this test object is used, the relation between $\Delta I/I$ and α , as I is varied, is one of simple proportionality. With other test objects, this relationship is more complicated.

Direct measurements of foveal visual acuity during anoxia have been made by McFarland and Halperin. During oxygen deprivation, as well as under normal

atmospheric conditions, the relation between visual acuity and intensity of illumination was in agreement with Hecht's equation. The effect of anoxia was a translation of the curve to the right on the $\log I$ axis just as in the case of $\Delta I/I$. Moreover, the average amount of this change was approximately the same as in the case of intensity discrimination for comparable degrees of anoxia. The similarity between the effect of anoxia on visual acuity and on intensity discrimination is further evidence that the two functions are related and suggests that they undergo parallel changes in the presence of other physiological stimuli, such as carbon monoxide or variations in blood sugar concentration.

In a limited sense changes in visual sensitivity may be considered an index of physiological imbalance of the organism as a whole. Such data, however, cannot be used to compare the effects of such stimuli as anoxia on one individual with those on another. There seems to be no correlation between the magnitude of threshold change and the severity of subjective symptoms for different subjects at any given oxygen tension. Nor is the change in visual sensitivity directly proportional to the degree of impairment of the organism as a whole. At simulated altitudes above about 16,000 feet, subjective complaints become rapidly accentuated with further ascent. The increase in threshold, on the other hand, rises less rapidly in this range of altitude than at lower ones (fig. 6). Therefore it cannot be assumed that if one physiological stimulus causes twice as great a change in threshold as is caused by another stimulus, then the organism as a whole suffers twice as severely. The relationship between the change in threshold and the general effect on the organism may be stated as follows: The change in threshold $[\Delta(\Delta I)]$ is a function of the magnitude of the physiological stimulus, such as oxygen tension (pO_2). The general effect on the organism (E) is also a function of the stimulus. Therefore, the general effect is a function of the threshold change. Stated in other symbols, $\Delta(\Delta I) = f_1(pO_2)$; $E = f_2(pO_2)$; $\therefore E = \phi[\Delta(\Delta I)]$. The function ϕ is probably not a linear one. No studies have been reported in which the effect of anoxia on any other psychological or physiological function was compared with the change in the visual threshold under the same conditions. Such studies would be necessary in order to determine the nature of the function ϕ .

The usefulness of measurements of this type may be illustrated in the following manner. In testing the efficacy of a given drug for counteracting the effects of anoxia, the changes in threshold may be measured during exposure to anoxia before and after administration of this substance. If the rise in threshold is reduced by one half after the drug is given, one cannot conclude that one half of the general effect on the organism has been counteracted. Unless the effect of the drug on vision is a specific one, the following conclusion might be drawn. The drug has reduced the effect of the oxygen tension employed and made it equivalent to an oxygen tension at which half the threshold rise ordinarily occurs. In order to determine this "effective altitude," one must refer to curves such as in figure 5, which must be determined separately for each subject. In experiments to be reported in subsequent papers of this series, visual thresholds were employed as an index in this way.

SUMMARY

1. The differential sensitivity of the human fovea was studied in relation to light intensity under a normal oxygen tension and during a constant degree of oxygen deprivation (10.8 per cent oxygen) in a low oxygen chamber. Final measurements were made while the subjects breathed 100 per cent oxygen through a nasal catheter. Nine subjects were used in these experiments.

2. The data are presented in two ways: *a*, as the differential threshold ΔI plotted against the intensity *I*, and *b*, as the ratio $\Delta I/I$ plotted against intensity. Both plots, on a double logarithmic grid, are fitted with theoretical curves derived from Hecht's equation.

3. The reduced oxygen tension resulted in a translation of the $\log \Delta I$ vs. $\log I$ curve to the right and upwards along a 45° asymptote. As the illumination increased anoxia caused progressively smaller changes in the threshold.

4. The effect on the $\log \Delta I/I$ vs. $\log I$ curve is a simple translation to the right. This means that to reach any given level of $\Delta I/I$ during a given degree of anoxia, the intensity *I* must be multiplied by a constant factor.

5. The mean magnitude of the shift during exposure to 10.8 per cent oxygen was 0.356 \log_{10} units. This is comparable to results obtained with other visual functions during equivalent degrees of oxygen deprivation.

6. The manner in which the effect of anoxia on differential thresholds varies with the degree of oxygen deprivation was studied on two subjects. The changes in visual sensitivity are plotted in relation to *a*, time; *b*, decrease in arterial oxygen saturation, and *c*, equivalent altitude, corresponding to the oxygen tension.

7. The increase of the differential threshold is initially proportional to the loss of arterial oxygen saturation but then tends to level off so that smaller increases result from further losses. The changes in the differential threshold plotted against equivalent altitudes are described by a sigmoid curve.

8. Changes in visual sensitivity are discussed as an index of physiological imbalance in the organism.

REFERENCES

- BARACH, A. L. *Anesth. and Analges.* 14: 79, 1935.
 BARTLEY, S. H. *Vision*. New York, D. Van Nostrand Co., Inc., 1941, p. 34.
 CROZIER, W. J. AND A. H. HOLWAY. *Proc. Nat. Acad. Sci.* 23: 23, 1937.
 J. Gen. Physiol. 22: 341, 1939.
 FISHER, R. A. *Statistical methods for research workers*. London, Oliver & Boyd, 1941, p. 117.
 GARRETT, H. E. *Statistics in psychology and education*. New York, Longmans, Green & Co., 1937, p. 223.
 GOLDSCHMIDT, S. AND A. B. LIGHT. *J. Biol. Chem.* 64: 53, 1925.
 GRAHAM, C. H. AND E. H. KEMP. *J. Gen. Physiol.* 21: 635, 1938.
 HECHT, S. *Proc. Nat. Acad. Sci.* 20: 614, 1934.
 HECHT, S. AND E. U. MINTZ. *J. Gen. Physiol.* 22: 593, 1939.
 HECHT, S., J. C. PESKIN AND M. PATT. *J. Gen. Physiol.* 22: 7, 1938.
 HECHT, S., S. SHLAER AND M. H. PIRENNE. *J. Gen. Physiol.* 25: 819, 1942.
 KELLER, M. *J. Exper. Psychol.* 28: 407, 1941.
 MCFARLAND, R. A. *Arch. Psychol.* 22: no. 145, 1932.
 J. Comp. Psychol. 23: 227, 1937.

- McFARLAND, R. A. AND J. N. EVANS. *Am. J. Physiol.* 127: 37, 1939.
- McFARLAND, R. A., J. N. EVANS AND M. H. HALPERIN. *Arch. Ophthal.* 26: 886, 1941.
- McFARLAND, R. A. AND M. H. HALPERIN. *J. Gen. Physiol.* 23: 613, 1940.
- McFARLAND, R. A., L. M. HURVICH AND M. H. HALPERIN. *Am. J. Physiol.* 140: 354, 1943.
- ROUGHTON, F. J. W. AND P. F. SCHOLANDER. *J. Biol. Chem.* 148: 541, 1943.
- SHLAER, S., E. L. SMITH AND A. M. CHASE. *J. Gen. Physiol.* 25: 553, 1942.
- SNEDECOR, G. W. *Statistical methods*. Ames, Iowa, The Iowa State College Press, 1940, p. 68.
- STEINHARDT, J. *J. Gen. Physiol.* 20: 185, 1936.
- WALD, G., P. V. HARPER, JR., H. C. GOODMAN AND H. P. KRIEGER. *J. Gen. Physiol.* 25: 891, 1942.

FATIGUE OF THE DEPRESSOR REFLEX

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The published evidence on fatigue of the central inhibitory process is scanty and contradictory. In 1912 Forbes presented data, obtained from studies of inhibition of the crossed extensor reflex in the decerebrate cat, which he interpreted as indicating fatigue of an inhibitory reflex. But Bayliss' work (1893, p. 314; see also 1924, p. 423) on the depressor reflex in the rabbit suggests that the inhibitory process does not fatigue. Bayliss stimulated the central end of the depressor nerve in the rabbit for seventeen minutes; the blood pressure fell to approximately one-half its normal level, remained at the low level throughout the entire period of stimulation, and returned to its original level upon cessation of the stimulation. This observation has been verified by Dempsey (1940) and in our present experiments. Dempsey stimulated the depressor nerve for as long as two hours and with frequencies as high as 120/sec. without obtaining a rise of blood pressure during this prolonged stimulation.

The present study of the depressor reflex was undertaken with the object of ascertaining whether the evidence which had been obtained from experiments on this reflex is adequate to prove that the inhibitory process cannot be fatigued.

METHODS. These experiments were performed on sixteen rabbits and six cats. Twelve rabbits were under dial anesthesia (Ciba, 0.5 to 0.65 cc. per kgm., intraperitoneally), and four rabbits were under urethane anesthesia (1.0 gram per kgm., in a 25 per cent solution, injected intraperitoneally). Four cats were anesthetized with dial (Ciba, 0.7 cc. per kgm., intraperitoneally) and the other two were under urethane anesthesia (1.0 gram per kgm., in a 25 per cent solution, injected slowly into the femoral vein after a preliminary etherization).

A tracheal cannula was inserted. Blood pressure was recorded, usually from the right carotid artery, with a Hürthle membrane manometer. The manometer system contained 6 per cent sodium citrate solution to prevent coagulation of the blood. Both common carotid arteries were tied, to eliminate the carotid sinus reflexes.

Shielded silver-wire electrodes were placed under the left depressor nerve in the rabbits and in those cats in which the depressor nerve was separate from the vagus nerve. In the other cats the electrodes were placed on the left vagus nerve. The usual procedure was to place the left vagus, the left cervical sympathetic, and the left depressor nerves all on one pair of electrodes, and to cut the sympathetic and vagus nerves centrally. All three nerves were always cut peripherally. The thin depressor nerve was thus protected from injury

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which might be involved in separating it for a sufficiently long space to place on electrodes, and from excessive drying. When handled in this manner, the depressor nerve remained in excellent condition throughout every experiment. The right depressor and vagus nerves were also routinely cut.

For obtaining pressor responses, shielded silver-wire electrodes were placed under the sciatic nerve, either right or left, in all the cats and in one rabbit. In the rest of the rabbits, shielded silver-wire electrodes were placed under the central end of the right vagus nerve. In these rabbits, the right depressor nerve was cut centrally to prevent any centripetal stimulation of it due to spread of the stimulus.

The electrical stimuli consisted of condenser discharges through a thyatron, which were passed through a transformer before application to the nerve. Two independent stimulators were used for applying stimuli simultaneously to two nerves.

PROCEDURE. Measurements of the inhibition of a reflex indicate the intensity of the inhibitory process only as long as the inhibition is incomplete. It is generally accepted that the fall of blood pressure which is produced by stimulation of the depressor nerve is caused mainly by the inhibition of the tonic discharge of vasoconstrictor impulses. Conceivably, the intensity of the inhibitory process which is produced by maximal stimulation of the depressor nerve may be more than sufficient to lower the blood pressure to the greatest extent that can be observed. Changes in the intensity of the inhibitory process can be demonstrated if the excitatory background is periodically augmented by eliciting pressor responses which are too large to be inhibited completely by stimulation of the depressor nerve. If fatigue of the inhibitory process does occur, its presence should be evident from the progressive changes in the partially inhibited pressor responses during continued stimulation of the depressor nerve.

The plan pursued in these experiments was to obtain at regular intervals a constant reflex rise of blood pressure by applying a definite stimulus to an afferent nerve, thus increasing periodically the excitatory background. After the constancy of the pressor response to the excitatory stimulus had been established, supramaximal stimulation of the depressor nerve was begun. The stimuli for obtaining pressor responses were repeated regularly, at intervals of one or two minutes, before, during, and after the period of stimulation of the depressor nerve. The periods before and after stimulation of the depressor nerve revealed any fatigue or other changes which might have occurred in the pressor responses. The changes in the pressor responses during the stimulation of the depressor nerve indicated changes in the intensity of the inhibitory process. The use of condenser discharges which had been passed through a transformer before application to the nerve rendered marked polarization unlikely, and the use of supramaximal stimulation for the depressor nerve made the results independent of changes in the excitability of the depressor fibers.

RESULTS. Difficulty was experienced in obtaining constant pressor responses in the rabbit. In some rabbits, stimuli applied to a sensory nerve

yielded only reflex falls of blood pressure, regardless of the intensity or frequency used. Considerable time was spent in finding satisfactory conditions of anesthesia and an afferent nerve suitable for obtaining reliable pressor responses. The sciatic nerve proved unsatisfactory for this purpose, although one rabbit had constant pressor responses to stimulation of the sciatic nerve. In general, it was easier to obtain a fall of blood pressure in the rabbit by stimulating a sensory nerve such as the sciatic than it was to obtain a rise of blood pressure. Sometimes, a rise of blood pressure resulted from the first stimulation, but every successive stimulation, regardless of the intensity used, produced a fall of blood pressure. Stimulation of the central end of the right vagus nerve in rabbits under dial anesthesia gave the most reliable pressor responses.

Stimulation of the depressor nerve produced a fall of blood pressure accompanied by excellent inhibition of the pressor responses. The blood pressure remained at the low level throughout the entire stimulation of the depressor nerve, and the pressor responses slowly increased. In other words, the inhibitory effect of stimulation of the depressor nerve upon pressor responses gradually decreased. The time required to demonstrate this impairment of inhibition varied greatly from animal to animal. In some animals, the impairment showed promptly after the first minute. In other animals, the impairment was extremely gradual and was not evident until after twelve minutes. The usual result was an impairment which was clearly present after six minutes.

That this impairment of the inhibitory effect was reversible was shown by the improvement of the inhibition when the stimulus was reapplied to the depressor nerve after a suitable pause. Figure 1 is a typical record in which impairment and recovery of the inhibition are both seen. The same qualitative results could be obtained from an animal a number of times during each experiment. Quantitative reproducibility was rare, however, mainly because of the difficulties in maintaining pressor responses which were constant over a sufficiently long period of time.

A period of from thirty minutes to an hour was required to demonstrate clearly an impairment of inhibition at one frequency. The preparation was not sufficiently stable with respect to pressor responses, blood pressure and level of anesthesia to permit comparisons of the time-course of the impairment of inhibition at different frequencies.

Table 1 summarizes the results obtained in these experiments.

In short, fourteen out of sixteen rabbits and four out of six cats showed an impairment of the inhibitory effect of stimulation of the depressor nerve upon pressor responses. In the two cats and the two rabbits in which an impairment of inhibition was not demonstrated, the pressor responses were very poor and declined progressively throughout the experiment; consequently, these experiments can hardly be considered valid.

DISCUSSION. The physiologist usually defines fatigue as a reversible impairment of performance because of previous activity. By reversible, he means that recovery occurs within a reasonable period of time. A reversible

impairment of the inhibitory effect of stimulation of the depressor nerve upon pressor responses has been demonstrated in these experiments. Because precautions were taken against polarization at the electrodes, because supramaximal stimuli were used, and because it is known that nerve axons fatigue very slowly in the frequency range studied in these experiments (frequencies equal to or

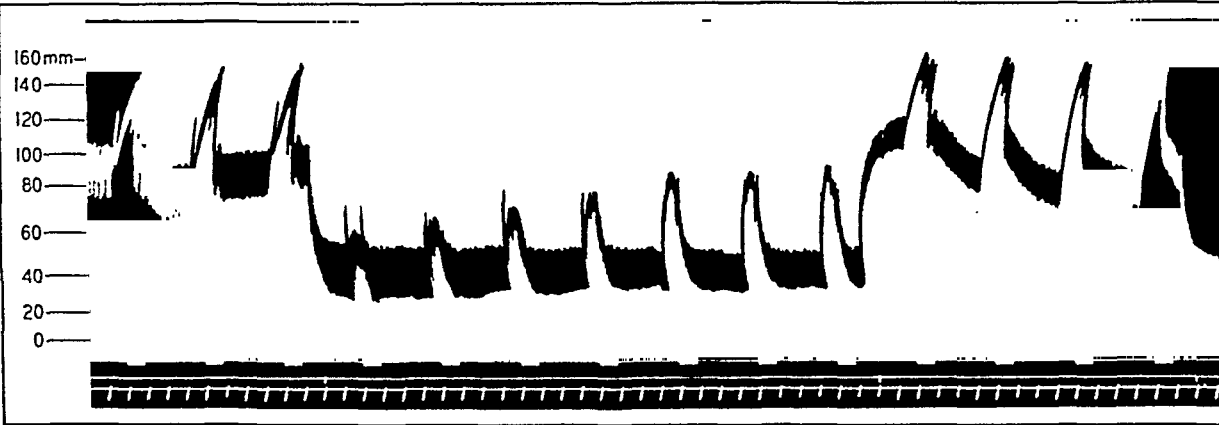


Fig. 1. Impairment of the inhibitory effect of supramaximal centripetal stimulation of the depressor nerve at 60/sec. upon regularly repeated pressor responses obtained by stimulation of the central end of the right vagus nerve at 60/sec. for 25 seconds. The stimuli were passed through a transformer before application to the depressor nerve as a precaution against polarization at the electrodes. Note the improvement of the inhibitory effect upon the pressor responses when the same stimuli were reapplied to the depressor nerve after a pause of eight minutes. Rabbit. Dial anesthesia. The upper signal line indicates stimulation of the right vague nerve. The left depressor nerve was stimulated between the first and second marks on the middle signal line and also after the third mark. Time in 30-sec. intervals.

TABLE 1

ANIMAL	ANESTHESIA	NERVE USED FOR OBTAINING PRESSOR RESPONSES	NUMBER OF ANIMALS	IMPAIRMENT OF INHIBITION EVIDENT
Rabbit.....	Dial	R. vagus	12	Yes
Rabbit.....	Urethane	R. sciatic	1	Yes
Rabbit.....	Urethane	R. vagus	1	Yes
Rabbit.....	Urethane	R. vagus	2	No
Cat.....	Dial	R. sciatic	2	Yes
Cat.....	Urethane	R. sciatic	2	Yes
Cat.....	Dial	R. sciatic	1	No
Cat.....	Urethane	R. sciatic	1	No

slower than 120/sec.), this impairment of the inhibitory effect of depressor stimulation is not peripheral, and hence, by exclusion, is central. These experiments have not eliminated Bayliss' argument (1924, p. 423) that a reversible impairment of inhibition may be due to fatigue of the *excitatory* process at a synapse presumed to occur in the interneuronal chain of synapses involved in the inhibitory reflex. The experiments show that the evidence which has been

obtained from studies of the depressor reflex is inadequate to prove that the inhibitory process cannot be fatigued.

SUMMARY

These experiments have demonstrated a reversible, central impairment of the inhibitory effect upon pressor responses of supramaximal, centripetal stimulation of the depressor nerve. Stimulation of the depressor nerve produced a fall of blood pressure which was accompanied by excellent inhibition of pressor responses. The stimuli for obtaining pressor responses were repeated regularly, at intervals of one or two minutes, throughout the experiment. The blood pressure remained at the low level throughout the entire stimulation of the depressor nerve, and the pressor responses slowly increased. In other words, the inhibitory effect of stimulation of the depressor nerve upon the pressor responses gradually decreased. This impairment was shown to be reversible and central; it illustrates fatigue of an inhibitory reflex.

It is concluded that the evidence which had been obtained from previous studies of the depressor reflex is inadequate to prove that the inhibitory process cannot be fatigued.

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REFERENCES

- BAYLISS, W. M. *J. Physiol.* 14: 303, 1893. Principles of general physiology. 4th ed., London, 1924.
DEMPSEY, E. W. Unpublished experiments, 1940.
FORBES, A. *Quart. J. Exper. Physiol.* 5: 149, 1912.

THE EFFECTS OF INFUSING GLYCIN AND OF VARYING THE DIETARY PROTEIN INTAKE ON RENAL HEMODYNAMICS IN THE DOG

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Glomerular filtration rate and renal blood flow in the dog are labile, varying from day to day with the dietary intake of protein (6, 12, 15, 18) and with the state of hydration of the animal (5, 14). However, if one chooses the experimental conditions of postabsorptive state and maximal hydration, filtration rate and blood flow remain quite constant over periods of several hours. Under these conditions intravenous administration of the amino acid glycine duplicates rapidly and reversibly those changes in the renal circulation which are seen over a period of hours following a protein meal (12, 13), and permits the ready investigation of the changes in renal vascular tone which serve as their basis. Utilizing methods for the quantitation of renal hemodynamics developed for the human (9, 10, 11, 16) and more recently applied to the seal (2, 8), we have observed that the increased filtration rate and blood flow following amino acid administration result from a fall in total renal resistance, which is predominantly localized in the efferent arteriolar and post arteriolar vascular components, although the afferent arterioles participate to a limited extent.

METHODS. Our experiments have been performed on two well trained female dogs under loose restraint. Adequate hydration was assured by the administration of 50 cc. of water per kilo body weight by stomach tube at the start of the experiment, and the intravenous administration of 0.9 per cent saline at a rate of 5 to 7 cc. per minute throughout the experiment. Creatinine, for the measurement of glomerular filtration rate, and sodium *p*-aminohippurate, for the measurement of minimum effective renal plasma flow, were dissolved in the infusions in amounts sufficient to ensure constant plasma concentrations of creatinine between 30 and 40 mgm. per cent and of *p*-aminohippurate between 1.5 and 3.0 mgm. per cent. Glycine was added to the infusions in increasing amounts to produce gradual increases in plasma concentration. Urine collection periods were 10 minutes in length and the bladder was washed out with distilled water at the end of each period. Arterial blood samples were obtained at the middle of each urine collection period from an indwelling femoral arterial needle fitted with a tight stylet. Mean arterial blood pressures were obtained by connecting a damped mercury manometer to the femoral needle for 2 minutes before and after the drawing of each blood sample. The mean of these two pressures, recorded on a kymograph, was taken to constitute the mean pressure for each experimental period. Only two of our dogs were sufficiently placid to give what we felt to be valid mean pressures by this method; hence the limitation of our experiments to two animals. Hematocrits were determined in the con-

ventional manner and chemical analyses were performed by the methods outlined in our previous communication (13).

Calculations. The clearance of *p*-aminohippurate is accepted as a measure of minimum effective renal plasma flow (3), having the same functional significance as the more widely used diodrast clearance. From the hematocrit and plasma flow¹ the minimum effective renal blood flow is obtained. Total renal resistance, plotted in figures 1 and 2, is calculated in absolute units of dynes cm.⁻⁵ sec. from the formula of Aperia (1).
$$R = \frac{P_m \times 1332}{R.B.F.}$$
 where R is the total renal per-

fusion resistance; P_m , the mean pressure; and $R.B.F.$, the renal blood flow per second. The virtue, if any, of expressing renal resistance in these units lies in the fact that recent studies of total peripheral resistance in the dog are expressed in these same units (17), and comparisons may readily be made.

In figures 4 and 5 total renal resistance, R_K , has been plotted in terms of millimeters mercury per cubic centimeter of blood per minute. Total resistance has been broken down into afferent arteriolar resistance, R_A ; efferent arteriolar resistance, R_E ; and postarteriolar resistance, R_V ; according to the equations of Lampert (11):

$$\begin{aligned} R_A &= \frac{P_m - P_{0'} - 23H_c - 20}{H \cdot D} \\ R_E &= \frac{(1 - 0.47F)(P_{0'} - P_0 - 23H_c + 10)}{H \cdot D} \\ R_V &= \frac{P_0 - P_V + 20}{H \cdot D} \\ R_K &= R_A + R_E + R_V \end{aligned}$$

P_m , mean pressure; P_0 colloid osmotic pressure of arterial plasma; $P_{0'}$, colloid osmotic pressure of plasma after the glomerular filtrate is formed; P_V , renal venous pressure; H_c , hematocrit; H , $\frac{1}{1 - H_c}$; D , *p*-aminohippurate clearance; F , filtration fraction. In order to apply these equations to our data it has been necessary to assume a serum protein of 7 grams per cent having an A/G ratio of 2.2, since this factor was not quantitated in our experiments.

RESULTS. *The effects on renal function of infusion of glycine in moderate amounts.* The consistent effect of the intravenous infusion of moderate amounts of glycine has been to increase the renal blood flow and glomerular filtration rate, and to decrease the filtration fraction (i.e., that fraction of the plasma entering the glomerulus which is filtered through the glomerular capillary walls). These changes, qualitatively similar in our two animals, have been somewhat greater in dog 1. The results of one typical experiment on this dog are shown

¹ The terms plasma flow and blood flow as used in the succeeding paragraphs are understood to have the connotation of minimum effective values. No correction has been applied for incomplete extraction, hence all flows are somewhat low. Since only comparative significance is attached to the data, incomplete extraction will not affect the interpretation.

in graphic form in figure 1. All variables, with the exception of plasma amino nitrogen concentration are plotted on logarithmic ordinates in order that equal deviations from the means may represent equal percentage variations. The first two experimental periods served to establish control values for each of the

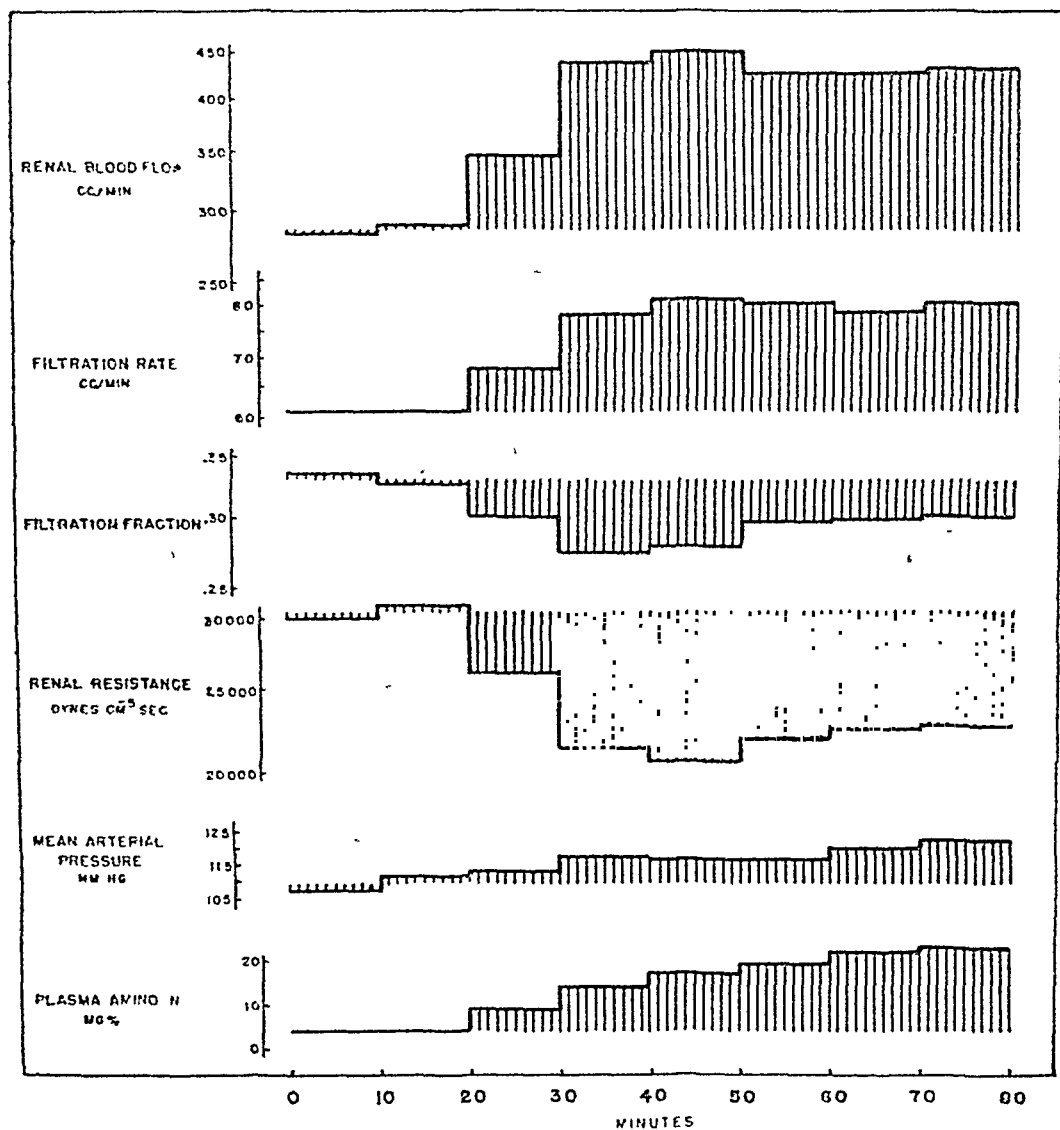


Fig. 1. The effects on renal function in dog 1 of intravenous infusion of glycine in amounts sufficient to raise the plasma amino nitrogen concentration to 22 mgm. per cent. All variables except amino nitrogen are plotted on logarithmic ordinates. The first 20 minutes establish control values for each of the variables. The glycine infusion was begun at 20 minutes and continued throughout the experiment, the plasma concentration progressively rising.

variables. The infusion of glycine was then begun and plasma amino nitrogen increased gradually from control values of 4.0 mgm. per cent to 22.8 mgm. per cent. Renal blood flow and glomerular filtration rate increased sharply, the former showing a greater percentage change than the latter, so that the filtration

fraction dropped. Little change occurred in the mean arterial pressure; thus the increased blood flow, of necessity, resulted mainly from the evident marked fall in renal resistance. Nine similar experiments yielded results in qualitative agreement with those presented in figure 1.

These changes in resistance and flow are not limited to the kidneys for uniformly the skin of the animal becomes warm and flushed. In the light of the well maintained pressure and barring some compensatory constriction elsewhere it may be assumed that an appreciable increase in cardiac output occurred. It is significant that in the human a sign of too rapid administration of amino acid with consequent increase in plasma concentration is a flushed warm skin.

The effects on renal function of infusion of glycine in toxic amounts. The administration of glycine at higher rates of infusion leads to the development of toxic signs of vomiting, dilatation and fixation of the pupils, weakness, muscular inco-ordination and eventual coma. Under these conditions renal blood flow and filtration rate fall, and filtration fraction rises as a consequence of increased renal resistance. The sequence of changes produced by elevating plasma amino nitrogen from 3.4 mgm. per cent to 61.0 mgm. per cent are shown in figure 2. In the early periods of this experiment during which plasma amino nitrogen levels were moderate (20 mgm. per cent or less) the changes in renal function previously described are evident. In the last two periods reversals of these changes are apparent, the renal blood flow falling considerably as a result of an increase in renal resistance. Filtration rate falls moderately so that filtration fraction increases. These results were duplicated in dog 2.

Comparison of renal hemodynamic changes in dog, man and seal. Changes in renal blood flow in the human, whether produced by the administration of pyrogens or adrenalin, are without effect on glomerular filtration rate. Consequently the filtration fraction is inversely related to renal plasma flow. According to Smith, Chasis, Goldring and Ranges (16) this is a result of the predominant control of renal blood flow by dilatation or constriction of the efferent glomerular arterioles.

Hiatt and Hiatt (8) studying the increase in filtration rate and blood flow in the seal which results from protein feeding, noted that the filtration fraction tends to remain constant. They conclude that in this form renal blood flow is regulated by variation in the calibre of both afferent and efferent arterioles. Our results on the dog seem intermediate between those on man and on the seal and to have elements in common with both. Thus an increase in flow is accompanied by a fall in filtration fraction as in man, and by an increase in filtration rate as in the seal.

The massed plot of our data, consisting of 96 clearance comparisons obtained in 12 such experiments as those described, are presented in figure 3. The filtration fraction (ratio of creatinine to *p*-aminohippurate clearance) is plotted against the renal plasma flow (*p*-aminohippurate clearance) expressed in cubic centimeters per square meter surface area per minute. Curve 1 is taken from the average data of Smith et al. (16) and curve 2 from that of Hiatt and Hiatt (8). The dashed curve is, by inspection, the best fit of a rectangular hyperbola

to the data obtained on the dog. No one of the curves fits our experimental results. The scatter of the data is much greater than may be explained by random error, and is felt to have physiological significance. However there is a suggestion of an inverse relationship between filtration fraction and plasma

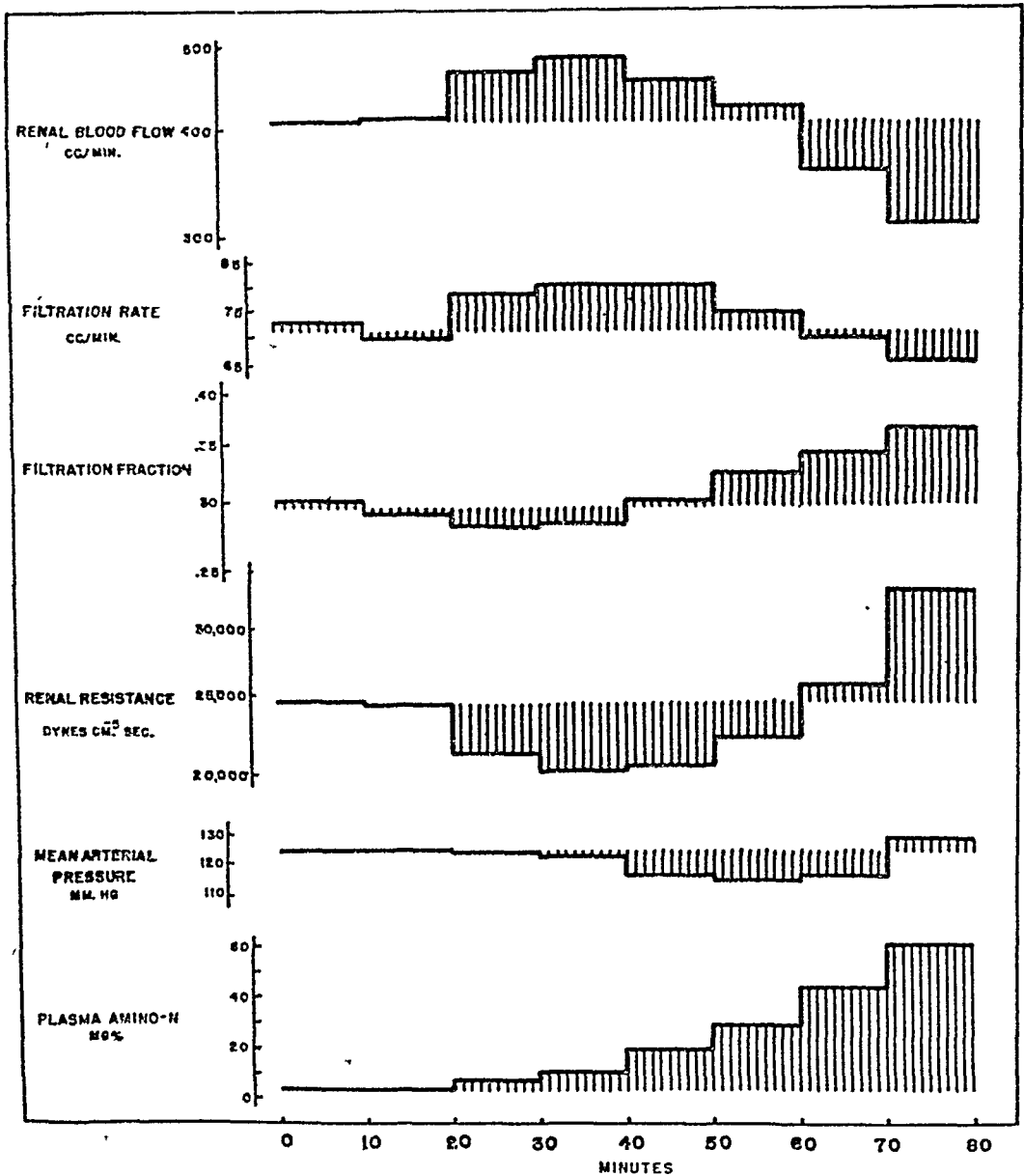


Fig. 2. The effects on renal function in dog 1 of intravenous infusion of glycine in amounts sufficient to raise the plasma amino nitrogen concentration to 61 mgm. per cent. Plotting of variables as in figure 1.

flow in the general grouping of points around the dashed curve. One might with some justification infer a predominant efferent arteriolar control of renal blood flow as in man, with added elements of afferent control, operating to a variable extent, as in the seal. The decrease in renal resistance, shown in fig-

ure 1 to result from glycine infusion, might therefore be assigned in large part to efferent arteriolar dilation.

The sites of change in renal vascular resistance. In an attempt to quantitate more exactly the site and relative degree of the resistance changes produced by moderate and high plasma amino acid levels, we have applied the revised equa-

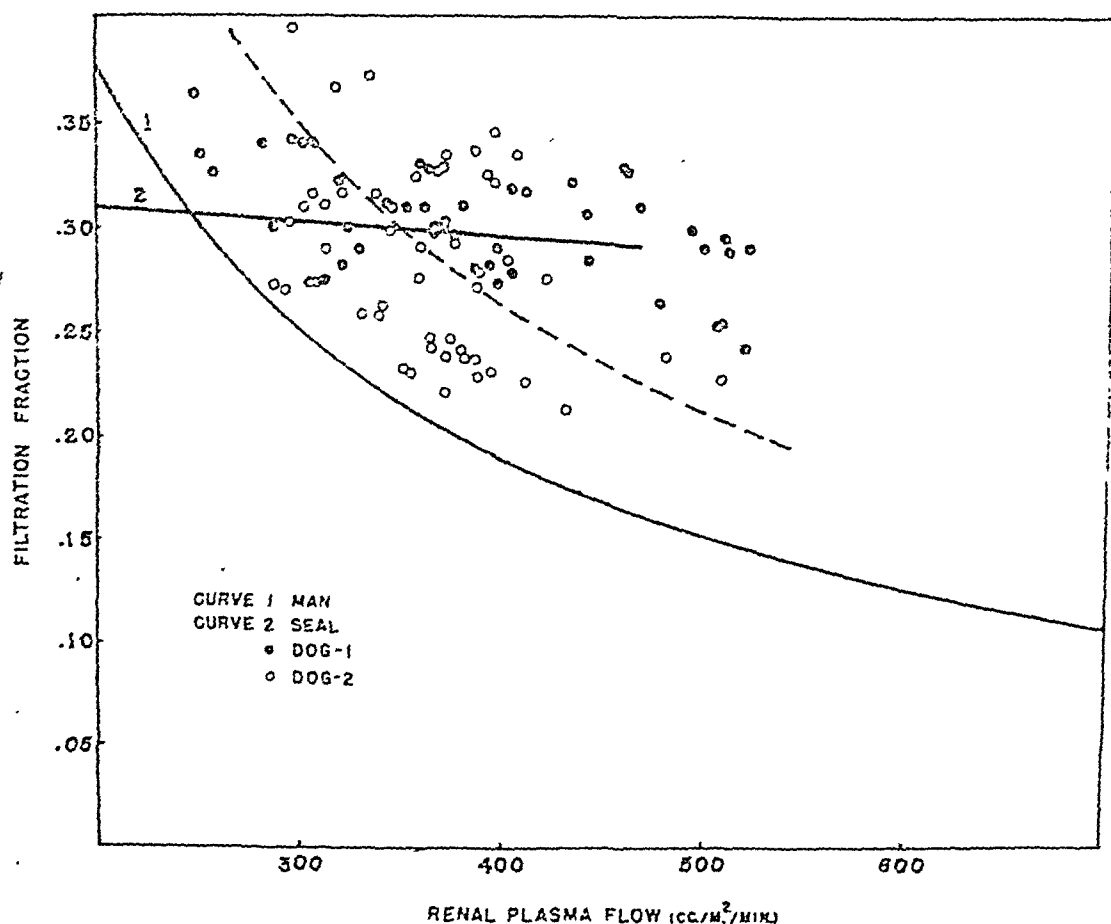


Fig. 3. The relation between filtration fraction and renal plasma flow in two dogs in which the plasma flow was varied by the infusion of glycine. Curve 1, from the data of Smith et al. (16) on man; curve 2, from the data of Hiatt and Hiatt (8) on the seal; dashed curve, by inspection, the best fit of a rectangular hyperbola to the data on the dog.

tions of Lampport (11) to our data². For a discussion of the significance and derivation of these formulae, which are given in the preliminary section on cal-

² The methods of treatment of data advocated by Smith et al (16) and by Lampport (11) do not necessarily lead to the same conclusions as to the relative importance of the afferent and efferent arterioles in the control of renal blood flow. In fact, when the data on man are analyzed by the two series of equations, opposite conclusions are reached. Each method of treatment depends on assumptions which have not been subjected to experimental verification and the author does not feel qualified to select between the two on their present merits. However, since the two methods give answers in essential agreement for the data on the dog, we feel justified in applying them although the discrepancies have not been resolved.

culations, the reader is referred to the original publications (9, 10, 11). In figure 4 are presented the changes in total renal resistance, R_K , produced by infusing moderate amounts of glycine in dog 1, and the breakdown of this resistance into the component afferent arteriolar, R_A ; efferent arteriolar, R_E ; and post arteriolar, R_V , resistances. Figure 4B is derived from the data of figure 1. Figure 4A is from another similar experiment on the same dog. The interesting factor in this analysis is the relatively insignificant changes in afferent arteriolar resistance and the predominant contribution of the efferent arteriolar and post-arteriolar resistance to the decline in total renal resistance. These changes were

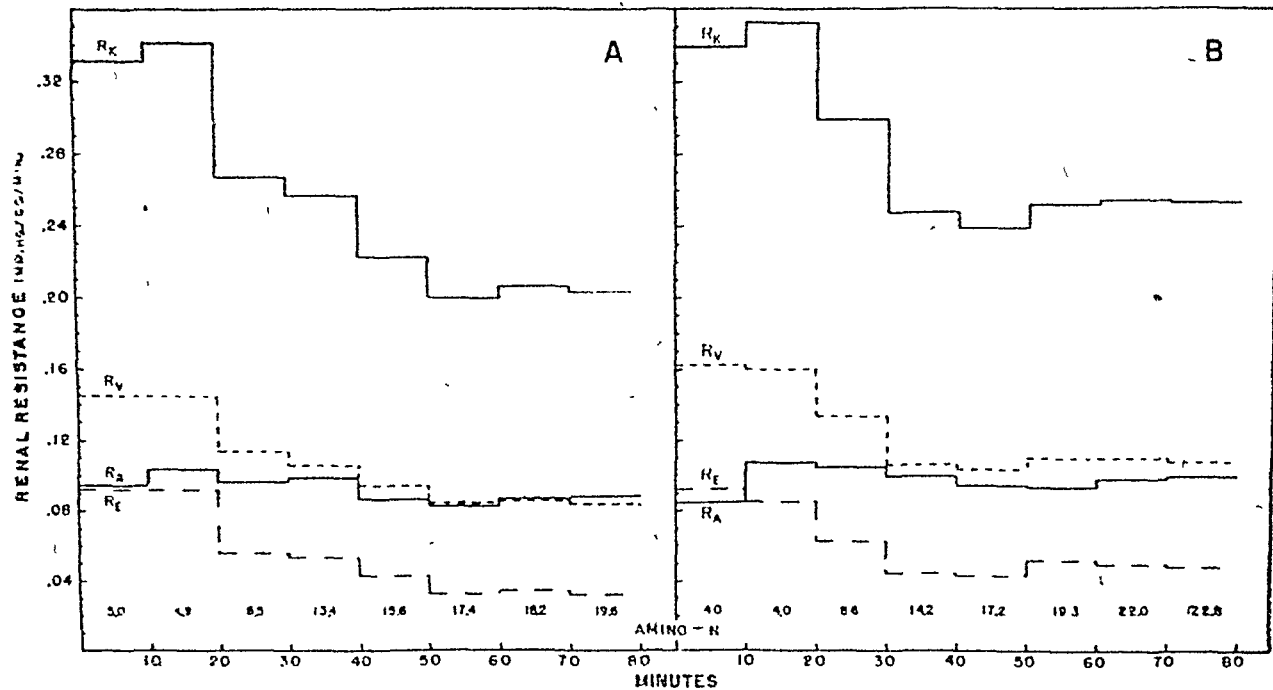


Fig. 4. The changes in the component resistances making up the total renal resistance of the dog produced by the intravenous infusion of amounts of glycine sufficient to elevate plasma amino nitrogen to from 19 to 22 mgm. per cent. R_K , total renal resistance; R_V , post-arteriolar resistance; R_A , afferent arteriolar resistance; R_E , efferent arteriolar resistance. A and B, two experiments on dog 1; B is calculated from the data of figure 1. The first 20 minutes of each experiment establish control values for each variable. The glycine infusion was begun at 20 minutes and continued throughout the experiment. Plasma amino nitrogen concentrations in milligrams per cent are given by the figures just above the abscissa.

qualitatively duplicated in dog 2 although they were of lesser magnitude. In some experiments a moderate decrease has been noted in afferent arteriolar resistance (see table 4, bottom row; and fig. 5A).

Figure 5A and B represent the resistance changes seen in experiments on dogs 1 and 2 in experiments in which toxic amounts of amino acid were infused. Figure 5A is derived from the data of figure 2. From these graphs it is apparent that all resistances contribute to the final elevation of total resistance seen in the final toxic state. In figure 5A there is in addition an appreciable fall in renal afferent resistance early in the experiment with moderate amino acid levels.

The analysis of the sites of change of renal resistance presented in figures 4 and 5 thus confirms in all essential features the inferences drawn previously from figure 3, namely, preponderant changes on the efferent side of the glomerulus coupled with changes in afferent arteriolar resistance of lesser degree.

The influence of maintenance diet on renal vascular resistance. In order to assess the changes in the separate renal vascular resistances as they are affected by variation in the protein content of maintenance diets, the dogs were placed for one week on each of three diets: a cracker meal diet (low protein), dog pellet diet (medium protein) and meat diet (high protein). Experiments similar to

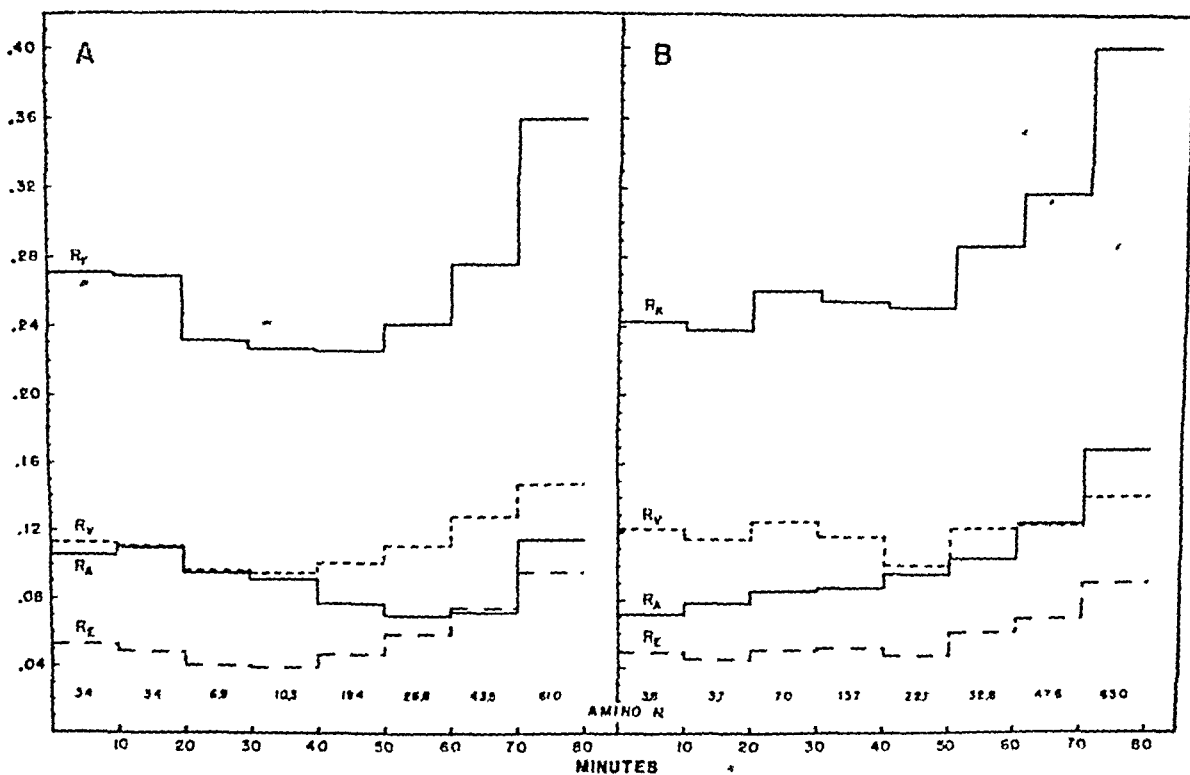


Fig. 5. The changes in the component resistances making up the total renal resistance of the dog produced by the intravenous infusion of amounts of glycine sufficient to elevate plasma amino nitrogen to from 61 to 63 mgm. per cent. Plotting of variables as in figure 4. A, an experiment on dog 1; B, an experiment on dog 2.

those of figure 1 were performed at the end of each week. All experiments were performed with the animals in a postabsorptive state and well hydrated. Results on dog 1 only are presented since this animal has consistently shown the more marked changes. Qualitatively the results on dog 2 are in agreement. In table 1 are presented, for each experiment on dog 1, the average values for the two control periods and for the two consecutive periods after amino acid which show the maximum functional change. By comparing the control periods, one sees the effects of variation in protein content of the maintenance diet. By comparing the amino acid periods one sees the effects of glycine infusion starting from three different functional base lines.

One may see from the control periods in table 1 that filtration rate and blood flow are reduced on carbohydrate maintenance diets and are elevated by increasing the daily intake of protein. Total renal resistance is higher on the carbohydrate regime than on the meat diet. The decreased resistance on the meat diet is evidently chiefly a function of reduction of efferent arteriolar and postarteriolar resistances, the afferent arteriolar resistance diminishing but slightly.

The effects of glycine infusion during each of the three dietary regimes is to reduce total renal resistance, the major component of this reduced resistance being localized on the efferent side of the glomerulus. On the meat diet there

TABLE I

The effect of variation in the protein content of the maintenance diet and of the infusion of glycine on renal function in the dog. In the column listing experimental procedure, the control periods refer to the 2 initial periods of each experiment prior to glycine infusion; the glycine periods refer to the 2 consecutive periods of maximal functional change during glycine infusion. In calculating the per cent change of renal resistances (last 4 columns) the values of the control periods on the carbohydrate maintenance diet serve as the standard of reference.

MAIN- TENANCE DIET	EXPERI- MENTAL PRO- CEDURE	NUMBER OF PERIODS AVERAGED	PLASMA AMINO-N	MEAN ARTERIAL PRESSURE	GLOMERULAR FILTRATION RATE	FILTRATION FRACTION	RENAL BLOOD FLOW	RENAL RESISTANCE				PER CENT CHANGE IN RENAL RESISTANCE			
								Afferent arteriolar	Efferent arteriolar	Post arteriolar	Total	Afferent arteriolar	Efferent arteriolar	Post arteriolar	Total
			mgm. %	mm. Hg	cc./ min.		cc./ min.	mm. Hg/cc. blood/min.							
Carbo- hydrate	Control	2	3.9	110	61.0	0.33	286	0.091	0.094	0.161	0.346	control	control	control	control
	Glycine	2	18.2	115	80.9	0.29	438	0.089	0.044	0.105	0.238	-3	-53	-35	-31
Mixed	Control	2	3.9	116	74.8	0.34	318	0.097	0.092	0.144	0.333	+7	-2	-11	-4
	Glycine	2	16.5	119	92.9	0.25	543	0.087	0.033	0.085	0.205	-4	-65	-47	-41
Meat	Control	2	3.4	116	86.1	0.33	372	0.089	0.075	0.124	0.288	-2	-20	-23	-17
	Glycine	2	16.2	115	108.0	0.30	508	0.073	0.047	0.091	0.211	-20	-50	-43	-39

is, however, an appreciable decline in afferent arteriolar resistance as a result of glycine infusion (20 per cent).

DISCUSSION. Hiatt and Hiatt (8) have explained the dietary variation in glomerular function in the seal as an adaptation to periodic food intake and limited availability of water. Since the seal derives its excretory water from its food, that water is most available for nitrogen and electrolyte excretion at the time food is being absorbed and actively metabolized. In interim periods glomerular function is reduced to conserve water. The same argument applies to a lesser extent to the dog, whose ancestors probably gorged at infrequent intervals and who were not always near adequate sources of water. In man there is evidence that glomerular filtration rate is but little affected by diet (4).

Perhaps the omnivorous ancestors of man were clever enough to ensure more frequent and regular meals and, by choice, lived near more abundant supplies of water. On such a basis the descending order of variability of glomerular function from seal to dog to man finds explanation in a decreasing need for adaptation for maximal nitrogen excretion with minimum expenditure of water. If the predominant efferent arteriolar control of renal blood flow in man, claimed by Smith et al. (16), is the factor maintaining the observed constancy of filtration rate, then the addition of progressively increasing variability of afferent arteriolar tone in the dog and seal permits the adjustment of filtration rate to the needs of the moment.

We have no evidence as to whether the changes in renal vascular tone produced by glycine are dependent on direct chemical effects on the several sites of renal resistance or are mediated through neural channels. The analogy of the action of pyrogens in producing equal increases of renal blood flow in innervated and denervated kidneys of the dog as demonstrated by Hiatt (7) might incline one to the former view. However, the widespread autonomic activity which is evident when toxic amounts of glycine are administered might well affect the renal circulation through the rich autonomic plexi.

SUMMARY

1. The intravenous administration of glycine in amounts sufficient to raise the plasma concentration to 20 mgm. per cent of amino nitrogen produces an increase in glomerular filtration rate and renal blood flow, and a decrease in filtration fraction and total renal resistance.

2. The decrease in total renal resistance is largely localized in the efferent arterioles and postarteriolar vascular bed; the afferent arteriolar resistance changes but slightly.

3. Glycine, in amounts sufficient to elevate the plasma concentration to 60 mgm. per cent of amino nitrogen, is toxic and produces a decrease in glomerular filtration rate and renal blood flow, and an increase in filtration fraction and total renal resistance.

4. The increase in total renal resistance results from an increase in each of the component renal resistances.

5. An increase in the protein content of the maintenance diet increases blood flow and filtration rate, without altering filtration fraction significantly, and decreases total renal resistance.

6. This decrease in total renal resistance is largely localized in vessels on the efferent side of the glomerulus.

REFERENCES

- (1) APERIA, H. *Skand. Arch. Physiol.*, Suppl. 16, 83: 1, 1940.
- (2) BRADLEY, S. E. AND R. J. BING. *J. Cell. and Comp. Physiol.* 19: 229, 1942.
- (3) FINKELSTEIN, N., L. N. ALIMINOSA AND H. W. SMITH. *This Journal* 133: 276, 1941.
- (4) GOLDRING, W., L. RAZINSKY, M. GREENBLATT AND S. COHEN. *J. Clin. Investigation* 13: 743, 1934.
- (5) HARE, R. S., K. HARE AND D. M. PHILLIPS. *This Journal* 140: 334, 1943.

- (6) HERRIN, R. C., A. RABIN AND R. N. FEINSTEIN. This Journal 119: 87, 1937.
- (7) HIATT, E. P. This Journal 136: 38, 1942.
- (8) HIATT, E. P. AND R. B. HIATT. J. Cell. and Comp. Physiol. 19: 221, 1942.
- (9) LAMPORT, H. J. Clin. Investigation 20: 535, 1941.
- (10) LAMPORT, H. J. Clin. Investigation 20: 545, 1941.
- (11) LAMPORT, H. J. Clin. Investigation 22: 461, 1943.
- (12) PITTS, R. F. J. Nutrition 9: 657, 1935.
- (13) PITTS, R. F. This Journal 140: 156, 1943.
- (14) SHANNON, J. A. J. Exper. Med. 76: 371, 1942.
- (15) SHANNON, J. A., N. JOLLIFFE AND H. W. SMITH. This Journal 101: 625, 1932.
- (16) SMITH, H. W., H. CHASIS, W. GOLDRING AND H. A. RANGES. J. Clin. Investigation 19: 751, 1940.
- (17) WIGGERS, H. C. This Journal 140: 519, 1944.
- (18) VAN SLYKE, D. D., C. P. RHOADS, A. HILLER AND A. ALVING. This Journal 110: 387, 1935.

CARDIOVASCULAR CHANGES RESULTING FROM SEVERE SCALDS

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Vascular microscopy shows that widespread vasoconstriction occurs in the mesentery, intestine, and ear during shock induced by burns, limb tourniquets and hemorrhage (1, 2). About one-half hour before death, gradual relaxation of the vessels occurs. Observations of renal blood flow also demonstrate severe vasoconstriction in the kidneys (Corcoran, Taylor and Page, 3). Since both a peripherally acting vasoconstrictor substance (Page, 4), other than angiotonin, and an angiotonin-like substance (Sapirstein, Ogden and Southard, 5; Hamilton and Collins, 6; Huidobro and Braun-Menendez, 7) have been demonstrated in the blood of shocked or burned animals, it is not unreasonable to assume that, at least in part, the vasoconstriction is due to one or both of these vasoconstrictors.

During these profound vascular changes there probably occurs physiologic and metabolic alterations in the muscles themselves of the blood vessels and the heart. To demonstrate some of these, we had recourse to previous work in which it was shown that refractoriness to the pressor action of such substances as angiotonin (9) and adrenalin (Rous and Wilson, 8) develop under a variety of circumstances in intact animals and to angiotonin, at least, in isolated perfused organs such as rabbits' ears (10). Examples of such are the refractoriness to angiotonin caused by injury to the nervous system, severe hemorrhage or shock induced by tourniquets. It was hoped, therefore, that a study of the effects of scalds on the vascular system by examination of the response of the blood vessels to pressor stimuli would demonstrate changes in the reactivity of the vascular and cardiac musculature.

It should be emphasized that in these experiments very severe scalds are inflicted to anesthetized animals in order better to demonstrate changes which might occur in the blood vessels and heart.

It would be doubtful that many such severely scalded patients would be seen clinically, and the fact that we were unable to save the animals with plasma does not militate against its beneficial effects in people less severely injured.

It would appear probable that, as in most severe burns, plasma is lost from the circulation but that the circulatory changes are wholly due to this is made doubtful by the fact that after severe hemorrhage the enhancement of the pressor response does not occur. It is only late after severe hemorrhage or after scalding that refractoriness occurs. Further, replacement of lost plasma by large amounts of reconstituted plasma or by continuous administration of saline failed to prevent wholly the typical cardiovascular responses to scalding. Encasement of the limbs in plaster to reduce local fluid loss also had no marked effect.

We have attempted to measure blood volume by the dye method in these severely scalded animals, but hemolysis is so extreme that even the newer dye methods, which aim to obviate this difficulty, failed.

METHODS. All experiments (176 animals) were done under pentobarbital anesthesia (35 mgm./kgm. of body weight given intraperitoneally). The animals were at all times well anesthetized. Survival was not sought. The hair on the legs was clipped short. A cannula was inserted into a carotid artery and connected to a mercury manometer with a tube filled with heparin solution. Both vagus nerves were severed in some experiments but not in others. Injections were made through a needle fixed in the external jugular vein or in the femoral vein. Before burning, single doses of adrenalin (0.1 to 0.8 cc. of 1:10,000), ephedrin (5 to 10 mgm.), pitressin (1 to 2 units), angiotonin 0.1 cc. pressor equivalent of 2 γ of adrenalin, tyramine (0.5 to 1 mgm.) or barium chloride (12 to 25 mgm.) were injected until reasonably constant pressor responses were elicited. Ephedrin and pitressin were soon discontinued because of their known ability to produce tachyphylaxis. Then all four legs of the dog were burned severely for two minutes, one at a time, by immersing in a bucket of water brought to the boiling point as in the method of Glenn, Peterson and Drinker (10a). The dog was again placed on its back and at intervals of about 10 to 25 minutes, single doses of the pressor drugs given. This was continued until death of the animal. In another group of animals only the hind legs were burned.

A number of procedures were tried in an attempt to modify the cardiovascular changes resulting from the burn. 1. Procaine solution (5-10 cc. 2 per cent solution) was injected into the cisterna magna after an occipital puncture. In these experiments just enough ether was given to allow the occipital puncture to be performed with ease and it was then discontinued when the effects of the procaine were apparent. No pentobarbital was used. Artificial respiration through a tracheal cannula was given throughout the experiment and more procaine administered if the animal started to breathe spontaneously. 2. Plaster encasements were applied in one group of experiments directly after the burn. The usual orthopedic plaster gauze was wrapped firmly around the limb and allowed to set. The application usually required from 5 to 10 minutes so that some swelling of the legs had already occurred before the plaster was rigid. Care was taken to include part of the lower abdomen in the casts. A number of other procedures were used but they can best be described along with their results.

RESULTS. The changes in the cardiovascular system studied by us resulting from burns may be divided into three phases: 1, the burn phase; 2, the transitional phase, and 3, the terminal phase (fig. 1).

The control period. The most striking observation made during this period was that the pressor response to adrenalin, tyramine, barium chloride, pitressin and angiotonin varied strikingly from animal to animal. In some dogs, 0.1 cc. of a 1:10,000 solution of adrenalin caused a rise of 80 mm. Hg or more while in others 0.8 cc. was required to produce a rise of only 30 mm. Hg. Nor was

the response to one drug a guide to that of others, though it was often true that a lively response to adrenalin or angiotonin was associated with an equally lively one to tyramine and barium chloride.

Depressor or biphasic responses to adrenalin were occasionally encountered. They could usually be overcome by giving repeated doses, larger than those producing the depressor response or by waiting for an hour or more.

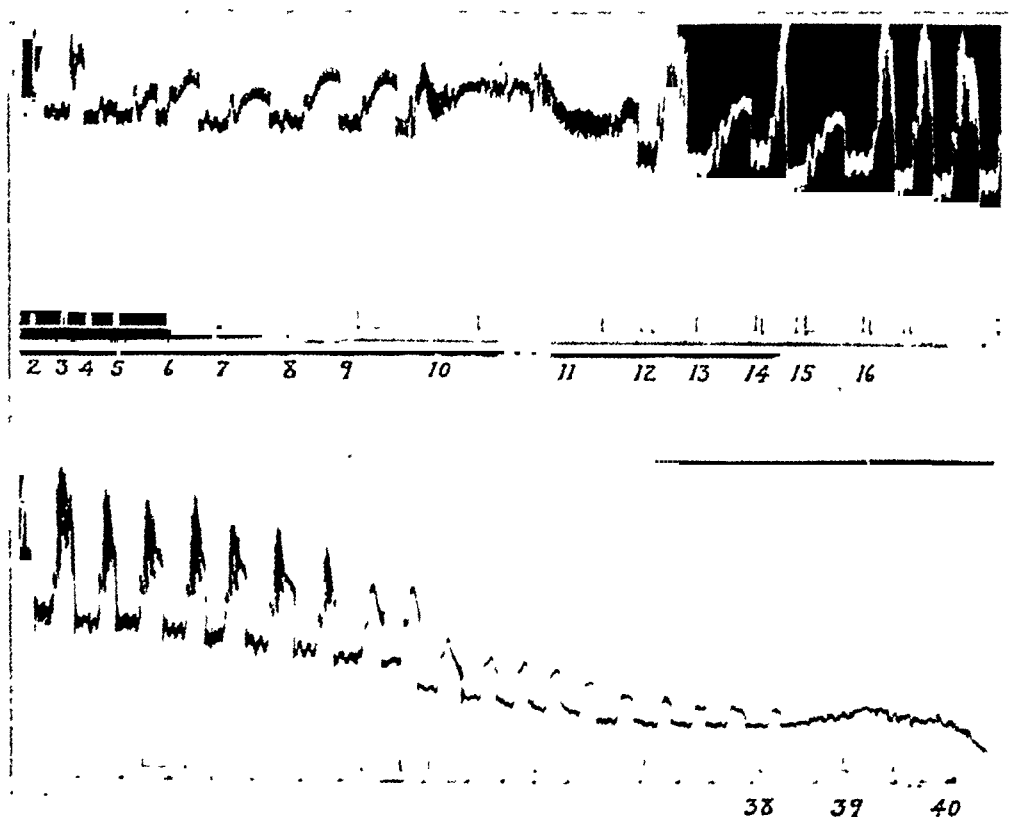


Fig. 1. The effect of burns on the vascular response of a dog to adrenalin and tyramine. No. 511 (2 and 3) adrenalin 0.3 cc. (1-10,000 dilution) (4) tyramine 1 mgm., (5 to 9) tyramine 2 mgm. (10 and 11) burned (12) adrenalin (13) tyramine (14) adrenalin (15) tyramine (16 to 38) adrenalin (39) tyramine (40) plasma infusion.

Four animals were tested for their pressor responses during an 8 hour period without inflicting a burn. In none of them was there any significant change. There was, therefore, no reason to believe that the experimental conditions without the burn were of themselves of such a nature as to bring on the hemodynamic changes to be described as the result of burns.

Ephedrin and pitressin were both used as test substances but both cause tachyphylaxis. The interpretation of the results was correspondingly difficult. The results of tests on 6 animals merely suggest that the response to ephedrin is reduced by burning and that pitressin is likely to cause a depressor rather than a pressor response.

In another four experiments, the depressor response to acetyl- β -methyl cho-

line (0.05 mgm.) was studied. When the animals became refractory to pressor drugs, mecholyl depressed the arterial pressure less actively than during the control period. Olson and Necheles (11) found the acetyl choline vasodepressor response inhibited by severe burns.

The burn phase. Immersion of a limb of an anesthetized animal into boiling water causes a sharp rise in arterial and intra-thoracic venous pressure and increase in rate and depth of respiration. This lasts 5 or more minutes followed by a return to the initial level. Occasionally a fall to much lower levels occurs within several minutes after the burn (table 1). When spinal anesthesia is used, no rise in pressure occurs but more usually a fall (expt. 5, table 1). This result is similar to that of Olson and Necheles (11) in which scalding a denervated foot caused a slight drop in arterial pressure while scalding of a normal foot caused a marked rise. The tissues of the limbs appear bloodless, gray and partially coagulated almost immediately after the burn but the flow of blood through the femoral vein is active. It should be noted, that the massive edema and swelling following application of tourniquets to the limbs was not observed. There is often a slight rise in hematocrit index from 15 to 20 minutes after the burn. For example, a rise of from 45 to 52 per cent might be seen. These rises do not always occur and are small in comparison with those we had expected.

Marked hemolysis of the blood occurs within 10 minutes as a result of the burn. The urine found in the bladder is usually deeply colored with hemoglobin.

The transition phase. Directly after the burn phase, the transitional phase begins. Vasoconstriction of the arteries, small and large, and veins occurs (Abell and Page, 2) and the plasma assumes vasoconstrictor properties almost immediately (4). A slight increase in swelling of the limbs may be observed and the hematocrit index may rise very moderately, show no further change, or tend to return to normal values. Visible edema of the legs is not marked.

Arterial pressure assumes a steady and usually reduced level and the heart beat is regular and forceful.

In many animals, directly after the burn, i.e., within 10 minutes, the pressor response to adrenalin and tyramine increases greatly but in a few the hyper-reactive phase was not marked. The increase is very striking in some experiments (fig. 1). Thus, in one example, the average response to adrenalin was 36 mm. Hg and 32 mm. Hg to tyramine before the burn. Twenty-five minutes after the burn, the response to adrenalin was 98 and to tyramine 56 mm. Hg. Three hours later the response to adrenalin was 150 mm. Hg. Most animals do not show such remarkable increase in sensitivity but this one serves to emphasize that during the transitional phase, increased responsiveness to pressor drugs is the rule and in many cases is startlingly great. Sensitivity may increase progressively during the first hour and the heightened response be maintained 3 hours or more. The transitional phase usually lasts from 3 to 4 hours and the limits are wide. The CO_2 combining power of venous blood is little reduced during this period, for example, 23.3 millimols per liter 3 hours after scalding.

The terminal phase. The terminal phase is initiated by beginning failure of pressor response to pressor and depressor drugs. No change in the average

TABLE 1

Examples of the pressor response to drugs before and after hot water burns in dogs

DOG NO.	TIME	PROCEDURE	PRESSOR RESPONSE TO								
			Adrenalin (1-10,000)			Tyramine			Barium chloride		
			Amt.	Initial B.P.	Rise in B.P.	Amt.	Initial B.P.	Rise in B.P.	Amt.	Initial B.P.	Rise in B.P.
			cc.	mm. Hg		mgm.	mm. Hg		mgm.	mm. Hg	
1	10:44	Burned	0.5	142	40	2	148	30			
	10:47		0.5	140	36	2	156	30			
	11:00										
	11:15		0.5	58	78						
	11:40		0.5	88	124	2	92	78			
	1:05		0.5	80	120						
	1:30		0.5	72	118	2	64	54			
	2:00		0.5	76	120	2	80	38			
	3:10		0.5	84	116	2	82	50			
	4:20		0.5	100	98						
	5:20		0.5	108	84						
	5:38		0.5	106	50	2	104	18			
	5:45		0.5	100	38						
	5:50		0.5	70	18						
	5:53		0.5	56	14						
	5:55		0.7	60	0						
	5:56		1.0	22	0						
	6:00	Dead									
2	9:41	Burned and plaster cast	0.2	156	46	1	192	38			
	9:44		0.2	160	42	1	198	32			
	10:13										
	10:45		0.2	106	50						
	11:15		0.2	116	66	1	120	34			
	10:20		0.2	94	36						
	12:40		0.2	74	50	1	68	44			
	1:15		0.2	66	44	1	64	44			
	1:50		0.2	60	18	1	66	2			
	1:52		0.2	60	18						
	2:00	Transfusion 250 cc.									
	2:06	Dead									
3	8:50	Burned and plaster cast	0.1	172	48	1	166	52			
	8:56		0.1	170	46	1	168	42			
	9:16										
	9:58		0.1	66	70	1	58	56			
	11:15		0.1	54	50						
	12:00		0.1	44	48						
	1:00		0.1	52	34						
	1:25		0.1	44	20						
	1:27		0.1	42	26						
	1:30		0.1	44	16						

TABLE 1—*Concluded*[illegible]

level of arterial pressure may occur or it may be much reduced. Nothing has been observed clinically which would indicate rapidly approaching death except progressive rise in intra-thoracic venous pressure and even this is not an early sign.

The loss of pressor response may be slow at first, requiring an hour or more for a reduction to one-half of the previous response, but then the rate is accelerated and it may be almost complete in 10 or 20 minutes. During, or shortly after this, arterial pressure begins to fall, at first gradually, then rapidly, ending in death in a few minutes. In short, when impaired response to pressor drugs begins, the terminal phase is initiated and death from vascular failure will soon occur. Angiotonin and adrenalin seem to be rather more sensitive indicators of this phase than tyramine. Barium chloride is less desirable as an indicator because it tends, after repeated doses, to elicit changes of rhythm in the heart beat.

Respiration alters but little until the very end when it becomes progressively more shallow (as measured by a pneumograph) and finally stops almost simultaneously with complete circulatory failure. Measurement of the intrathoracic venous pressure in 34 of the experiments shows that a significant rise occurs when death is imminent.

Analysis of the blood pressure records shows no consistent relationship between the height of the arterial pressure and the response elicited from any of the pressor drugs.

When the response to adrenalin is lost, or all but lost, administration of 10 times the test dose failed to elicit more than a slightly greater response than the lower dose. It appears, therefore, that refractoriness is all but complete and that increasing the intensity of the stimulation does not overcome it.

Cardiometer studies. It seemed desirable to learn whether the heart as well as the blood vessels failed to respond to pressor stimuli during the terminal phase of circulatory failure resulting from burns. To this end Dr. Kenneth Kohlstaedt and Mr. Clifford Wilson have conducted a number of experiments with the dog's heart in a cardiometer. One typical experiment, the results of which are illustrated in figure 2, will be cited to exemplify the results. After each dose of adrenalin administered before burning, the arterial pressure (I on the record), the intrathoracic venous pressure (II on the record) rose and the volume of the heart (III on the record) fell. The enhanced response after scalding is seen in both the blood pressure and cardiometer record in section C, injection 2. The venous pressure now falls after administration of adrenalin. Sections E and F show the diminution and final obliteration of the vascular and cardiac response. The record of the animal's death is given in section G; arterial pressure falls to zero, venous pressure rises sharply and the heart dilates.

The Effect of Hemorrhage on the Pressor Response to Adrenalin. If reduction in blood volume were the sole cause of the hemodynamic effects of scalds, then the same phenomena should be observed after severe hemorrhage. Six experiments were performed in which the arterial pressure was severely reduced by hemorrhage and the response to adrenalin observed. An example is given in table 2.

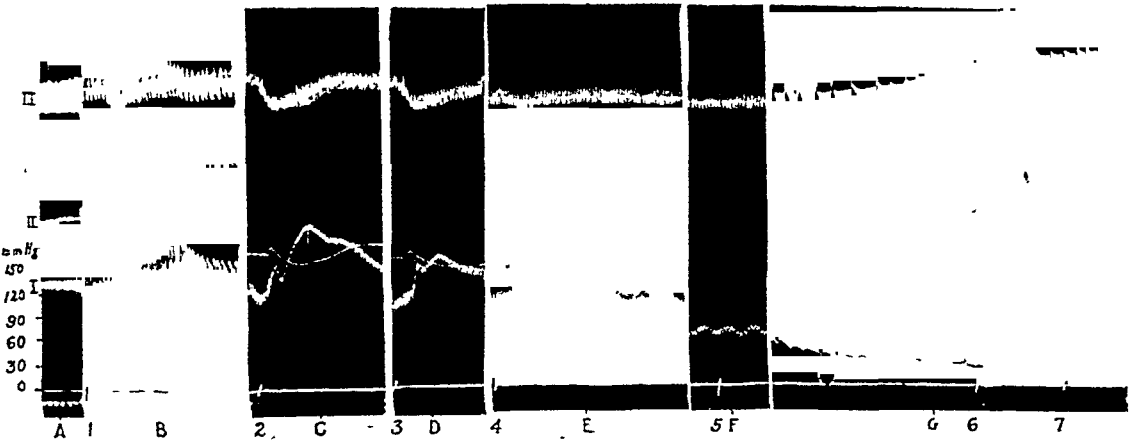


Fig. 2. I. Arterial pressure measured in carotid artery by a mercury manometer. II. Intra-thoracic venous pressure measured by a water manometer. III. Cardiac output measured by cardiometer. Cardiometer used at normal intrathoracic pressure. Lower border is systolic volume. Upper border of tracing is the diastolic volume.

Section A: Control period—drum moving slowly.

Section B: Control period—drum moving fast. 1, 0.4 cc. 1-10,000 adrenalin.

Section C: 1 hour and 10 minutes after abdomen, back and hind legs were burned. 2, 0.4 cc. 1-10,000 adrenalin.

Section D: 50 minutes elapsed between C and D. 3, 0.4 cc. 1-10,000 adrenalin.

Section E: 3 hours after D. 4, 0.4 cc. 1-10,000 adrenalin.

Section F: 10 minutes after E. 5, 0.5 cc. 1-10,000 adrenalin.

Section G: 15 minutes after F. 6, 0.4 cc. 1-10,000 adrenalin. 7, 0.4 cc. 1-10,000 adrenalin.

TABLE 2

The effect of hemorrhage on pressor response to adrenalin

Male dog weighing 15.4 kgm.

PROCEDURES	HEART RATE PER MIN.	STROKE VOLUME	RISE IN B.P. AFTER ADRENALIN	TOTAL BLOOD LOSS	TIME	INITIAL B.P.
		cc.		cc.	min.	mm. Hg
Control.....	150	11.3				
40 γ adrenalin.....	130	12.8	26			82
Control.....	150	11.4				
After bleeding.....	130	9.3		200	0	
40 γ adrenalin.....	110	11.3	18			70
After bleeding.....	130	7.7		300		
40 γ adrenalin.....	120	9.2	24			
After bleeding.....	130	6.9		380	23	58
40 γ adrenalin.....	120	8.6	24			50
After bleeding.....	140	6.1		410	38	
40 γ adrenalin.....	150	8.1	18			48
After bleeding.....	160	6.6		450	52	
40 γ adrenalin.....	170	6.9	14			46
After bleeding.....	160	5.7		500	62	
40 γ adrenalin.....	160	6.1	8			46
After bleeding.....	80	6.6		510	68	
40 γ adrenalin.....	130	6.9	2			44

With each dose of adrenalin, stroke volume increases when the arterial pressure rises, but as the responsiveness to pressor action of adrenalin decreases, so also does the extent of the rise in stroke volume decrease. It will be noted that directly after hemorrhage, no enhancement of the pressor response to adrenalin occurs as is so common after scalds but, just as in scalds, the response progressively diminishes until none is observed, despite the fact that the change in arterial pressure is not great. Thus at a pressure of 50 mm. Hg the response to adrenalin was 24 mm. Hg and stroke volume increased from 6.9 to 8.6 cc. At 46 mm. Hg the response to adrenalin was 8 mm. Hg and stroke volume rose from 5.7 to 6.1 cc.

Prolonged oligemia clearly is associated with vascular refractoriness, but does not account for the enhancement of the response observed after severe scalding. The question also remains just what the association is between loss of blood volume and production of refractoriness.

Attempts to modify the effects of scalds. The major portion of this investigation was carried out in the early spring and midsummer. Thirty-five control experiments showed an average span of life after scalding of 5 hours with extremes of 2 and 9 hours. A smaller part of the work was done in the fall, and for some unexplained reason, 17 control dogs survived an average of 40 hours, again to fall to the 5 hour period the next spring. For this reason, whenever possible, a control experiment was carried out on the same day as the main experiment.

The attempts to modify the circulatory effects of scalds were of varied nature and since they were for the most part unsuccessful, they will not be presented in detail.

a. Use of plaster encasements on the limbs to limit loss of plasma—18 experiments with an average survival time of 4 hours as opposed to 5 hours in the control group.

b. Heparin (1,760 Connaught units)—3 experiments, survival 4.5 hours.

c. Soluble vitamin K (10 doses totalling 18.5 mgm. after burning)—3 experiments, survival 5 hours.

d. Ascorbic acid (0.12 mgm./cc. infused 10 drops a minute throughout experiment) 1 experiment, survival 3 hours.

e. Sodium succinate (0.1 gram/cc., 1 to 2 cc. per minute infused; total about 60 grams) 5 experiments, survival 6 hours, control 5 hours.

f. Procaine (injected into cisterna magna, artificial respiration) 11 experiments, survival 4 hours.

g. Nicotinic acid (5 to 16 grams as infusion) 3 experiments, survival 7 hours.

h. Methionine (3 grams as infusion) 1 experiment, survival 9 hours.

i. Transfusion whole blood (100 to 300 cc. citrated dog's blood) given early after burning may enhance the pressor responses. Late in the terminal phase may precipitate death. Six experiments, survival 7 hours.

j. Concentrated human albumin (0.2 cc./min., 36 to 240 cc.) 6 experiments, survival 7 hours.

k. Human plasma (1 to 2 cc./min., 240 to 620 cc.) 4 experiments, survival 4.5 hours.

- l. Acacia (7 per cent 1 to 3 cc./min.) 7 experiments, survival 7 hours. Refractoriness seemed to develop more slowly.
- m. Sino-aortic denervation. Five experiments, survival 4 hours.
- n. Adrenalectomy (1 to 3 days before burning, 5 to 10 mgm. cortate daily) 4 experiments, survival 1 hour. Pressor responses normal before burning.
- o. Bilateral nephrectomy (2 days before burning) 24 experiments, survival 6 hours.
- p. Pitthng—4 experiments, survival $3\frac{1}{2}$ hours.
- q. Refrigeration (limbs packed in ice after burning) 3 experiments, survival $6\frac{1}{2}$ hours.
- r. Intraperitoneal saline (1 cc./min.) 5 experiments, survival 9 hours, controls 8 hours.
- s. Intravenous saline (1 to 3 cc./min., total 800 to 2000 cc.) plaster encasements did not affect mortality in 5 experiments. In 10 experiments survival was 9 hours compared with 5 hours for controls. In a second group the animals were immersed up to the sternum and burned area immediately placed in a cast. Nine control animals survived 28 hours while the saline treated ones lived 38 hours.

DISCUSSION. Cardiovascular responses to severe scalding allow three phases to be clearly differentiated. The first of these, occurring while the burn is being inflicted and blocked by spinal anesthesia—the burn phase—is characterized by a sharp rise in arterial pressure followed by a fall. The second—the transitional phase—is characterized by enhancement of pressor responses and moderate fall in arterial pressure. The third—the terminal phase—is heralded by an initial slow and then ever more rapid loss of response to pressor drugs. When the pressor response is significantly reduced, vascular collapse may occur at any time. Increasing the intensity of the chemical stimulus does not overcome the cardio-vascular refractoriness.

The heart and the blood vessels both appear to participate in these changes in responsiveness, and suggest that the muscle of the heart and blood vessels is altered rather than some change at the myoneural junction. Greatly strengthening this view is the fact that the response is changed to such diverse substances as adrenalin, tyramine, angiotonin, barium chloride and choline derivatives. Burning must have profoundly altered the metabolism of the muscle of the vascular system as expressed by a hyperactive phase followed by what appears to be a phase of exhaustion.

Hemorrhage also alters vascular reactivity to angiotonin (9, 12) and adrenalin (8, 12), but here no hyper-reactive phase is discernible, only the refractory phase. Rous and Wilson (8) rejected slowing of circulation and severe vasoconstriction as a cause of refractoriness. The response to adrenalin is enhanced early in traumatic shock in cats to be reduced terminally according to Freedman and Kabat (13). Injury to the nervous system produces alterations in responsiveness. For example, Cannon and Lyman (14) showed that pitthng cats enhanced the pressor action of adrenalin. Traumatic injury to the nervous system in dogs and cats reduces or abolishes the pressor response to angiotonin according to

Page (9). Whether we are to view the changes resulting from burns as belonging to this category is uncertain. Against this is the fact that spinal anesthesia or pithing does not abolish them. This argument is not wholly conclusive.

Visual study of the vessels in animals burned in the same manner as that employed in these experiments showed severe constriction during the transitional phase and relaxation during the terminal phase (Abell and Page, 2). If, therefore, two effects of adrenalin, i.e., vasoconstriction and vasodilatation, are attributed to opposite actions depending upon the state of the muscle—contraction when relaxed, relaxation when tonically shortened (Cannon and Lyman, 14), then just the opposite of our results should have been observed.

Since the vessels are partially relaxed when the terminal phase occurs, it would seem improbable that vasoconstriction was sufficiently intense to reduce or abolish the effect of adrenalin.

It might be suggested that there was so little blood in the arterial portion of the vascular system that contraction of the arterioles would have little influence on arterial pressure. But inspection of the vessels under the microscope shows them to be well filled with blood. Further, this view does not explain the failure of the myocardium to respond.

Anoxia might well be responsible for the changes in vascular responsiveness observed in the terminal phase, but this does not as readily explain why the responses should be enhanced during the transitional phase. In the absence of data to prove the point we shall merely mention it as a possibility.

The carotid sinus mechanism does not appear to play an important part in the cardiovascular changes resulting from scalds, for sino-aortic denervation does not prevent them from occurring. It might be argued that the lowered blood pressure would mean increased vasoconstriction due to reduced sinus and aortic depressor nerve action, hence pressor drugs would have less constrictor effect since vasoconstriction is already maximal. This view has several defects. The first is that in the terminal phase, arterioles tend to dilate rather than to constrict. This has been seen with the microscope. The second is that the heart becomes refractory just as do the blood vessels, as shown by the cardiometer studies. The third is that a sharp fall in blood pressure due to brief hemorrhage is not accompanied by a reduced adrenalin response in an anesthetized but otherwise normal animal. The fourth is that sino-aortic denervation does not significantly change the response nor does it prolong the life of the animal.

During the terminal phase transfusion seems to hasten rather than retard death. A number of observers have pointed this out giving a variety of reasons why this is so. To these may possibly be added the factor of loss of responsiveness of the vascular system to humoral agents.

Hemolysis which is well known to occur as the result of burns (15) might play some part but the fact that refractoriness develops after a prolonged period of hypotension due to hemorrhage or tourniquet shock in which little or no hemolysis occurs suggests that it is not decisive.

Since vasoconstriction is one of the constant accompaniments of burn shock,

except during the terminal phase, it is natural to seek a cause for it. Reduction of blood volume would lead to passive reduction in the caliber of the vascular bed. But the fact that, for example, in the kidneys vasoconstriction persists long after blood volume is restored (Corcoran, Taylor and Page, 3) points to the vasoconstriction as being an active one. Furthermore, it occurs even in denervated kidneys which leads one to suspect the presence of humoral vasoconstrictors. Such substances have been demonstrated in the plasma of shocked animals (4).

Thus it is a possibility that some substance is liberated in scalded animals which increases the excitability of the vascular and cardiac musculature during the transitional phase, the continued action of which leads to exhaustion (terminal phase). It might be the vasoconstrictor substance which we have described as appearing in the blood during burns and shock. Possibly it is the substance liberated in the blood and lymph from the burned area which increased the QO_2 of rat liver slices described by Muus and Hardenbergh (16). Like the vasoconstrictor, it also is ultrafiltrable.

Attempts to modify the various phases of the circulatory excitation and depression during burns have met no marked success. Limiting the loss of plasma by plaster casts, altering the coagulability of the blood by heparin, vitamin C or K, sodium succinate, nicotinic acid, methionine, procaine anesthesia of the brain and cord, refrigeration of the burned limbs, bilateral nephrectomy and destruction of the cord were all of little avail. Adrenalectomy so reduced the resistance of the animals to the burns that it was not possible, under the conditions of our experiments, to examine satisfactorily the response to pressor drugs. There can be no doubt that removal of the adrenal glands even when followed by administration of desoxycorticosterone seriously reduced the chances of survival after burning. Maintenance of blood volume with blood but especially with saline seems to postpone the terminal phase and prolong survival to a limited degree.

SUMMARY

1. Severe scalding modifies the circulation in dogs under pentobarbital anesthesia in such a way that at first the pressor responses to such drugs as adrenalin, tyramine, and to a lesser extent, angiotonin and barium chloride, are enhanced, to be followed by a period of refractoriness.

2. It is possible to divide the cardiovascular responses to scalding into three phases: 1, the burn phase; a short phase while burning is actually occurring, during which arterial pressure rises and all the phenomena associated with acute nervous excitation occur; 2, the transitional phase during which arterial pressure tends to fall slightly or moderately and response to some pressor drugs is enhanced; 3, the terminal phase during which the response begins to fail and may be abolished altogether. Increasing the intensity of the chemical stimuli does not overcome the cardiovascular refractoriness.

3. Not only the peripheral vascular bed becomes refractory, but the heart as well, as cardiometer studies have shown.

4. Severe acute hemorrhage early produces neither enhancement nor depression of the adrenalin response in the heart or peripheral vessels. For this and other reasons, it is assumed that the cardiovascular changes may be an indirect rather than a direct effect of oligemia.

5. The sino-aortic nerve mechanism does not appear to play an important part in the circulatory changes after scalding.

6. Maintenance of blood volume and administration of saline tend to postpone slightly and shorten the terminal refractory phase but do not abolish it. Salt solution possibly prolongs life somewhat.

7. Transfusion given during the terminal phase may hasten death.

8. The suggestion is tentatively made that these results may be due to the appearance or disappearance of substances which control the excitability of the vascular and cardiac musculature. Heightened excitability may lead ultimately to exhaustion and the terminal refractory phase.

REFERENCES

- (1) PAGE, I. H. AND R. ABELL. *J. Exper. Med.* 77: 215, 1943.
- (2) ABELL, R. J. AND I. H. PAGE. *Surg., Gynec. and Obstet.* 77: 348, 1943.
- (3) CORCORAN, A. C., R. D. TAYLOR AND I. H. PAGE. *Ann. Surg.* 118: 871, 1943.
- (4) PAGE, I. H. *This Journal* 139: 386, 1943.
- (5) SAPIRSTEIN, L. A., E. OGDEN AND F. D. SOUTHARD. *Proc. Soc. Exper. Biol. and Med.* 48: 505, 1941.
- (6) HAMILTON, A. S. AND D. A. COLLINS. *This Journal* 136: 275, 1942.
- (7) HUIDOBRO, F. AND E. BRAUN-MENENDEZ. *This Journal* 137: 47, 1942.
- (8) ROUS, P. AND G. W. WILSON. *J. Exper. Med.* 29: 173, 1919.
- (9) PAGE, I. H. *J. Exper. Med.* 78: 41, 1943.
- (10) PAGE, I. H. AND O. M. HELMER. *J. Exper. Med.* 71: 495, 1940.
- (10a) GLENN, W. W. L., D. K. PETERSON AND C. K. DRINKER. *Surgery* 12: 685, 1942.
- (11) OLSON, W. H. AND H. NECHELES. *This Journal* 139: 574, 1943.
- (12) KOHLSTAEDT, K. G. AND I. H. PAGE. *Arch. Surg.* 47: 178, 1943.
- (13) FREEDMAN, A. M. AND H. KABAT. *This Journal* 130: 620, 1940.
- (14) CANNON, W. B. AND H. LYMAN. *This Journal* 31: 376, 1912-13.
- (15) SHEN, S. C. AND T. H. HAM. *New England J. Med.* 229: 701, 1943.
- (16) MUUS, J. AND E. HARDENBERGH. *J. Biol. Chem.* 152: 1, 1944.

THE RESPONSE OF MAMMALIAN SMOOTH MUSCLE TO OXYGEN AT HIGH PRESSURE AND ITS POSSIBLE RELATIONSHIP TO OXYGEN POISONING OF RESPIRATORY ENZYME SYSTEMS

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The similarity of the responses of isolated smooth muscle (longitudinal duodenal muscle of rabbit) to cyanide, to oxygen at high barometric pressures, and to low oxygen, has recently been demonstrated (Bean and Bohr, 1940). This was interpreted as an indication that an oxidative dysfunction was responsible for the reaction of the smooth muscle under each of these conditions, since cyanide is known to inactivate selectively the oxidase-cytochrome enzyme system; oxygen at high pressure has been shown to decrease the activity of the dehydrogenase system (Libbrecht and Massart, 1937; Bohr and Bean, 1940), while low oxygen administration grossly deprives the tissue of oxygen without directly damaging these enzymes.

Interestingly enough, however, a few early experiments on pyloric sphincter tissue showed that it does not react in an identical manner to each of these oxidative disturbances. This peculiarity seemed to warrant further investigation and in the experiments herein reported attempts were made to determine whether such differences in the reaction of the pyloric sphincter to cyanide and to high oxygen pressure might not be correlated with the modes of action of these two respiratory enzyme poisons and to uncover some of the more intimate details of the mechanism by which oxygen at high pressure poisons the dehydrogenase system.

The smooth muscle used was taken from freshly killed rabbits and in most cases was prepared and set up in a compression chamber in the manner previously described (Bean and Bohr, 1940); in a few instances the more usual method for studying smooth muscle at atmospheric pressure was employed. Particular care was taken to maintain the temperature (37°C.) and the mechanical stimulation inherent in the bubbling of the bath with oxygen, constant. Although it is believed by some (Johnson, McCloskey and Voegtlin, 1927) that in guinea-pig uterus the necessary energy for contraction is not dependent upon the supply of molecular oxygen, it appears generally accepted that the processes responsible for the maintenance of tonus in smooth muscle are ultimately if not immediately dependent upon oxidative processes (Evans, 1919; Garry, 1923). In our experiments we have used changes in tonus as a criterion of the action of the enzyme poisons in question.

Effects of high oxygen pressure. Curve A, figure 1, represents the typical change in tonus which occurred without exception when the pyloric sphincter was exposed to oxygen at seventy-five pounds' gauge pressure. The tonus com-

menced to fall within three minutes after compression and reached a new equilibrium at a lower level. This change was rapidly and almost completely reversed by decompression to atmospheric pressure. That this reversible tonus change was due to the increased O_2 pressure and not to some effect of pressure *per se* was shown by the fact that in control tests sphincter tonus was not altered by exposure to room air at seventy-five pounds pressure. The tonus fall induced by the high oxygen pressure is, perhaps, best explained by what amounts to a "hyperoxic anoxia"—a failure of utilization of oxygen due to the poisoning or

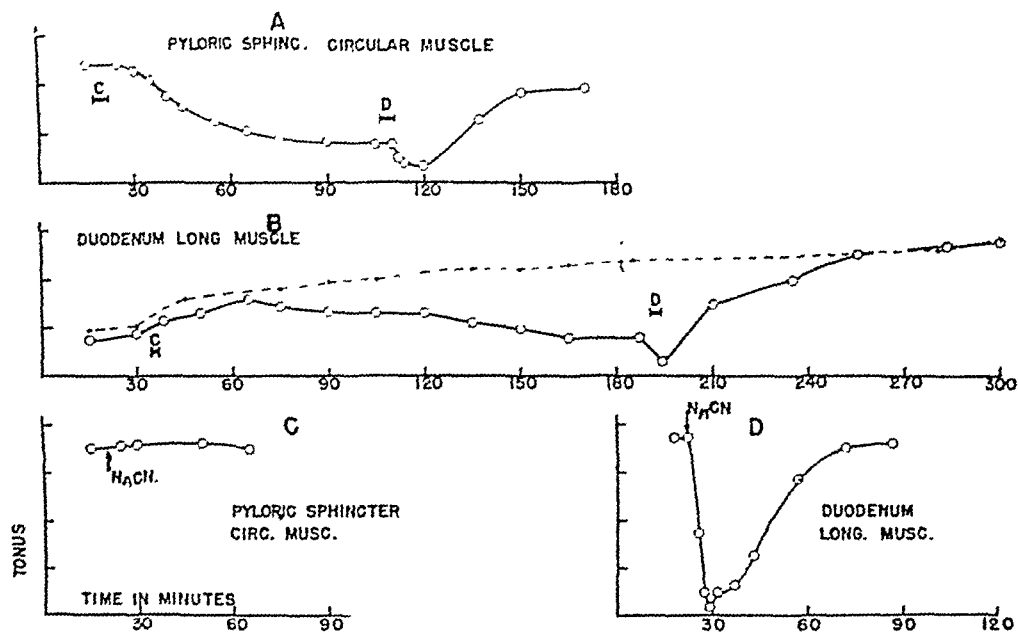


Fig. 1. A. Changes in tonus of circular muscle of pyloric sphincter (rabbit) induced by exposure in Tyrode solution to oxygen at 75 pounds' gauge pressure. Compression at C, decompression to atmospheric pressure at D. Tonus on the ordinates indicates relative values. Time in minutes on the abscissae.

B. Tonus changes in longitudinal duodenal muscle. Broken line indicates tonus of control exposed in Tyrode solution to oxygen at atmospheric pressure; the solid line, changes induced by oxygen at 75 pounds' pressure;

C. Tonus of pyloric sphincter in Tyrode to which $NaCN$ was added to give a final concentration of 1:100,000.

D. Tonus change induced in fresh longitudinal duodenal muscle by $NaCN$ (concentration 1:100,000).

inactivation of the dehydrogenase system by oxygen in high concentrations (Bohr and Bean, 1940).

The curve B, figure 1, showing the effects of oxygen at seventy-five pounds' pressure on longitudinal duodenal muscle, is reproduced for comparison. The tonus changes induced in a control strip of this tissue by exposure to oxygen at atmospheric pressure (broken line) and those induced in a test strip by exposure to oxygen at seventy-five pounds' pressure (solid line) were recorded simultaneously. It is seen that the tonus of the control and test preparations followed the same gradient for thirty-five minutes, after which the tonus of the test prepara-

tion decreased; on decompression to oxygen at atmospheric pressure the tonus of the test strip returned to and continued along with the gradient of the control.

A comparison of curves *A* and *B* of figure 1 clearly indicates that the tonus of both pyloric sphincter and longitudinal duodenal muscle is reversibly decreased by exposure to oxygen at high pressure. But a point deserving emphasis is the striking difference in the time of onset of this depressant action in the two muscles. In the pyloric sphincter it invariably appeared within five minutes after compression, whereas in the longitudinal muscle it was delayed until anywhere from fifteen to forty minutes in each of a series of ten experiments. This difference in the onset of depressed tonus would appear to justify the conclusion that either the high-oxygen-sensitive enzyme system does not play as important a rôle in the maintenance of the tonus of longitudinal duodenal muscle as it does in the pyloric sphincter, or the enzyme system responsible for the maintenance of tonus in the longitudinal duodenal muscle is more slowly damaged by oxygen at high pressure.

It cannot be said with absolute certainty that intrinsic nerves play no part in the induction of the above described tonus changes of isolated pyloric sphincter and longitudinal duodenal muscle, but the fact that such changes are not eliminated by previous atropinization of the tissue offers reasonable assurance that the parasympathetic endings do not act as intermediaries; nor do the sympathetic endings appear to be involved (Bean and Bohr, 1940).

Effects of cyanide. It is generally accepted and has been again demonstrated (Dale, 1937) that cyanide acts only as an asphyxial agent preventing the utilization of oxygen and does not exert any peculiar toxic influence on tissues; the effects of its administration in our experiments, therefore, can be quite safely attributed solely to a deficient oxygen utilization. Sodium cyanide added to the Tyrode bath to give a final concentration of 1:100,000 or even as high as 1:9000 failed to alter sphincter muscle tonus (*C*, fig. 1). Such concentrations are far in excess of that required to inactivate the oxidase-cytochrome enzyme system either *in vitro* or *in vivo* (Evans, 1919; Hadidian and Hoagland, 1939). The failure of NaCN to alter sphincter tonus indicates that this oxidative enzyme system is not essential for the maintenance of tonus in pyloric sphincter muscle.

In contrast to this apparent absence of any cyanide action on the tonus of pyloric sphincter muscle is the pronounced depressant action of NaCN on the tonus of longitudinal duodenal muscle. In some few experiments this drop was preceded by a slight initial transient rise which was seemingly conditioned by the freshness of the tissue since it was missing in freshly isolated tissue and became more prominent with prolongation of the preservation of the tissue in the ice box up to 48 hours (fig. 2). But even in isolated tissue preserved for 6 days this initial NaCN elevation was transient, the final result of cyanide administration being a decrease in tonus.

In attempting to explain this initial increase in tonus especially prominent in older tissue a number of possibilities come to mind. It has been convincingly demonstrated for a number of tissues (Gesell, Brassfield and Hamilton, 1942; Brassfield and Gesell, 1942) that potentiation of acetylcholine by increased

acidity is an exceedingly important determinant in the physiological response of tissues and it is conceivable that the initial increase in tonus of longitudinal duodenal muscle sometimes induced by the administration of cyanide might be interpreted as an acid potentiation of acetylcholine liberated from intrinsic nerve endings. But this would seem to be ruled out by the finding that heavy atropinization of the tissue previous to the cyanide administration did not prevent the occurrence of this initial increase in tonus. Moreover, after two days—to say nothing of six—the cut cholinergic fibers would, in all probability, have degenerated (Cannon and Rosenblueth, 1937).

The fact that increasing the pH of the bath by small amounts of NaOH will of itself induce a rise in tonus of the duodenal muscle constitutes further evidence against the interpretation that the initial tonus rise induced by cyanide might be due to acid potentiation of acetylcholine and suggests that such initial rise

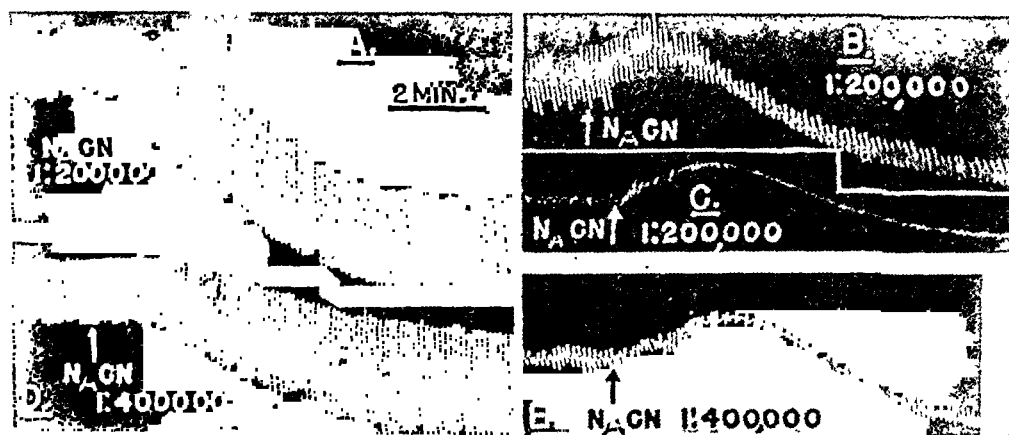


Fig. 2. A. Effect of NaCN, 1:200,000 on fresh rabbit duodenum (longitudinal muscle). B. Effects two days after isolation (tissue from the same animal as in A). C. Six days after isolation. D. Effect of NaCN, 1:400,000 on fresh rabbit duodenum. E. Effects of NaCN on a similar strip of tissue from the same animal as in part D but two days after isolation of the tissue.

might actually be the result of an increased pH of the bath arising from the NaCN which of itself is strongly alkaline. Alkalinization of the bath by the NaCN was ruled out as a possible explanation by the finding that adjusting the pH of the NaCN to that of the bath by adding of HCl just before its administration failed to eliminate the initial increase in tonus.

Another possible explanation worthy of consideration involves the question of glycolysis. It is quite conceivable that there might be an alteration in glycolysis and some alteration in the normal co-ordination of the respiratory enzyme systems with perhaps a partial shift to anaerobic mobilization of energy following the tissue's removal from the body. In this connection the work of Dale (1937) on the frog heart is of interest; he found that any shortage of oxygen appears to induce it to break down carbohydrate and, what is not true of the heart under aerobic conditions, to stimulate it to utilize the carbohydrate in the perfusion fluid. Now if the conditions are at all comparable in the isolated duodenal tissue it may be that the initial rise in tonus elicited in our experiments by cyanide

represents a temporary shift to a utilization of the glucose of the Tyrode solution. In order to test if this were the case, a comparison of the reaction to cyanide before and after poisoning the tissue with iodoacetic acid was made. Some difficulty was encountered in getting a concentration of I.A.A. which of itself would not completely depress spontaneous activity of the tissue, but it was found that after immersing the tissue in Tyrode solutions with I.A.A. in concentrations of 1:30,000 for thirty minutes, cyanide administration elicited only a drop in tonus. The initial rise in tonus present in unpoisoned muscle but which was missing in the I.A.A. poisoned muscles would, therefore, appear to be at least partially related to an alteration in glycolysis resulting from a shift in enzyme activity which becomes more pronounced as the tissue's isolation from the body is prolonged. Incidentally, the increased sensitivity of smooth muscle after sympathetic and parasympathetic denervation (Cannon and Rosenblueth, 1937) might very possibly be a result of a comparable shift in enzyme co-ordination.

The typical precipitous drop in tonus induced by cyanide (NaCN , 1:100,000) administered to strictly fresh longitudinal duodenal muscle is shown in curve *D*, figure 1. This substantiates the conclusion that a cyanide-sensitive enzyme is essential for the maintenance of tonus in this tissue. The possibility that this characteristic drop in tonus of fresh longitudinal duodenal tissue induced by cyanide might have been due to some peculiar liberation of acetylcholine in large "paralytic" amounts from the parasympathetic endings was ruled out by the fact that when, during such a state of lowered tonus, acetylcholine was administered, a sharp contraction was elicited.

In brief then, the evidence thus far presented indicates that pyloric sphincter tonus is sharply decreased by exposure to oxygen at high pressure, but is unaltered by concentrations of cyanide which inactivate the oxidase-cytochrome system. On the other hand, the tonus of the longitudinal duodenal muscle is distinctly less rapidly depressed by oxygen at high pressure, but relatively weak concentrations of cyanide induce a precipitous fall in tonus. These differences in the effects of the selective enzyme poisons on the tonus of these two tissues in their fresh state strongly suggest that the oxidative metabolism of the two tissues is controlled by two fundamentally different enzyme systems. The integrity of the high-oxygen-sensitive system, involving in all probability a dehydrogenase, would seem to be essential for the maintenance of tonus in the slowly acting pyloric sphincter muscle, whereas in the more rapidly acting longitudinal duodenal muscle the maintenance of tonus is largely dependent upon a properly functioning oxidase-cytochrome system. If we assume equivalent rates of penetration, the delayed drop in tonus in the longitudinal muscle induced by oxygen at high pressure might very well represent the inactivation of a dehydrogenase system which is playing a secondary rôle in the maintenance of tonus in this muscle. However, as will be pointed out further along in this report, the possibility that this delayed fall in tonus could also represent a delayed inhibitory action of the high oxygen pressure on the oxidase-cytochrome system must be recognized.

The fact that in the normal intact animal the longitudinal muscle is possessed

of a relatively rapid rhythmic action whereas the sphincter is a slower, tonically-contracting tissue, and also that these two tissues are not affected in the same manner or degree by their autonomic innervations might provide a justifiable basis for expecting that the oxidative mechanisms of the two tissues are different and that differences in the response of the tissues to selective enzyme poisons as described above might very well be anticipated. In this connection the findings of Hadidian and Hoagland (1939) regarding the relative speeds of action of the two enzyme systems in question are of interest. The cytochrome system, which they found to be the faster step in their double enzyme system, is the one which, as our experiments indicate, controls the oxidations in the more rapidly acting longitudinal duodenal muscle; the slower dehydrogenase system governs oxidations in the sluggishly acting sphincter tissue.

The experimental data and the interpretation that the tonic and contractural activity of these two tissues is controlled through different enzyme systems have broad implications; they point to the probability that the neurohumoral substances effective on the tissues operate through their influence on these enzyme systems. The difference in the effects of acetylcholine on longitudinal duodenal muscle (stimulation) and on the pyloric sphincter (inhibition) are particularly pertinent in this connection; the difference may simply be a reflection of the ability of this neuro-humoral substance to make specific enzymes available for the necessary energy transformations. Certainly the fact that acetylcholine not only inhibits the pace-maker of the cold-blood heart, but also renders the cardiac muscle reversibly unresponsive to electrical stimuli and reduces the resting metabolism to one-half of its normal value (Clark, 1935) might well be interpreted as being due to the action of acetylcholine on an essential enzyme system whereby it is rendered unavailable for oxidative processes; on the other hand, in tissues where this neuro-humoral stuff excites them to increased activity, the more important enzymes necessary for energy mobilization would be those whose availability is greatly increased by acetylcholine. It has already been pointed out (Raper, 1940) that an increased uptake of oxygen resulting from the addition of acetylcholine to slices of submaxillary gland tissue is due neither to an increase of substrate, nor to the supply of oxygen, so that the most apparent explanation for the increased oxygen uptake is an increase in the availability of the catalyst.

Effects of high oxygen pressure and cyanide in combination. Pyloric sphincter tissue: In this series of experiments changes in tonus were again used as a criterion of the toxic action of oxygen at high pressure and thus indirectly, also, for the inactivation of the dehydrogenase enzyme systems. The tonus of the pyloric sphincter tissue, as pointed out above, is not altered by the administration of cyanide so that any changes in tonus which might occur when cyanide and oxygen at high pressure are administered in combination may be safely attributed to an involvement of oxygen.

The results of three successive exposures of the same strip of sphincter muscle to high oxygen pressure are shown in figure 3, which represents the invariable findings obtained under the specified conditions. A typical fall in tonus (curve A) was induced by the first exposure to oxygen at thirty pounds' pressure. This

effect was partially reversed by decompression to atmospheric pressure. Preceding the second exposure to oxygen (curve *B*, fig. 3) cyanide was added ("NaCN") to the bath in amount sufficient to give a final concentration of 1:20,000; the tonus, which at that time was decreasing very slightly, was not significantly altered. Although this cyanide treatment was without any obvious effect it did change conditions in the tissue, for a subsequent exposure to O₂ at thirty pounds' pressure (*C*) induced a rise in tonus instead of a fall as had occurred in O₂ exposures before cyanide administration (curve *A*). This rise persisted for about

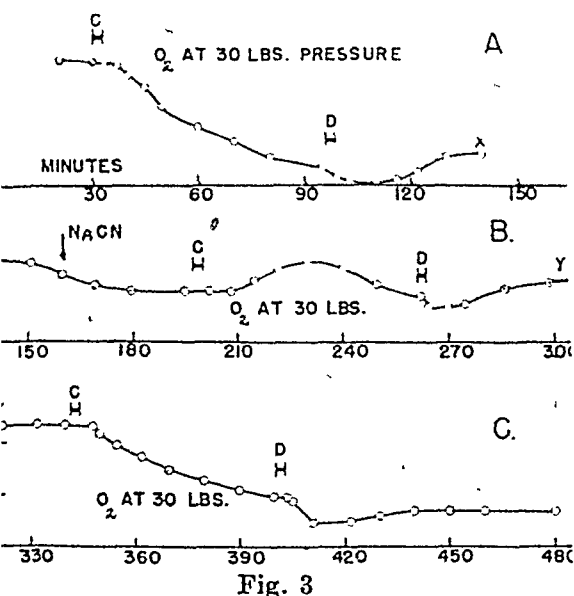


Fig. 3

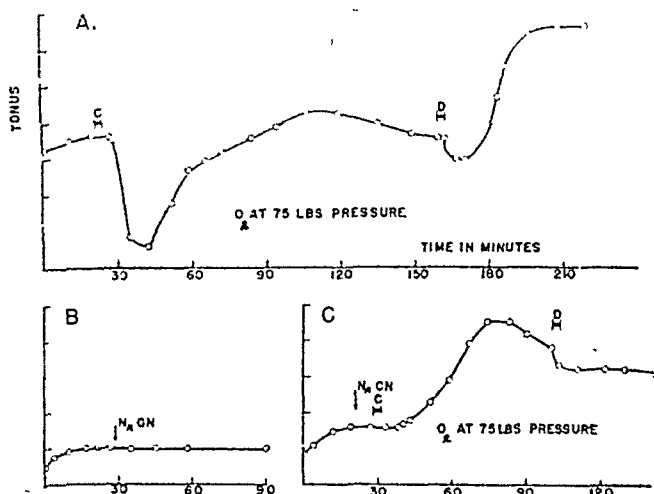


Fig. 4

Fig. 3. Tonus changes induced in a strip of circular muscle of pyloric sphincter (rabbit) in Tyrode bath: *A*. By exposure to oxygen at 30 pounds' gauge pressure. *B*. By exposure to oxygen at 30 pounds' pressure after having previously administered NaCN to give a bath concentration of 1:20,000. *C*. By exposure to oxygen at 30 pounds' pressure in a fresh Tyrode bath after having thoroughly washed the tissue to remove the NaCN. In each curve *C* indicates compression; *D*, decompression to atmospheric pressure and the arrow at NaCN in curve *B* marks the moment of administration of cyanide.

Fig. 4. Tonus changes induced in circular duodenal muscle. *A*, by oxygen at 75 pounds' pressure; *B*, by NaCN in final concentration of 1:15,000; *C*, by oxygen at 75 pounds following cyanide administration. The sharp tonus drop immediately following decompression shown in this and figures 1 and 2 was due in part to the buoyance of minute bubbles collecting on the surface of the tissue and cannot therefore be considered as a tonus change.

thirty minutes and was followed by a secondary drop. Shortly after decompression (*D*) the tonus returned almost to the precompression level. The tissue was then thoroughly washed to remove the cyanide and placed in a fresh bath of Tyrode solution; subsequent exposure to oxygen at thirty pounds' pressure (*C* of curve *C*) induced a depression of tonus similar to that recorded before any cyanide had been administered (curve *A*). In another series of similar experiments it was found that oxygen at seventy-five pounds' pressure produced essentially the same results as those shown in figure 3 except that the tonus increase induced by the oxygen at the higher pressure after cyanide administration was more pronounced.

These results, taken together, would indicate then that cyanide administered to isolated pyloric sphincter temporarily nullifies the deleterious action of oxygen at high pressure on this tissue, and, since cyanide poisons the more important systems which provide "activated" oxygen, it may be that the deleterious action of oxygen at high pressure in the absence of NaCN is due to the presence of *unduly great* amounts of "activated" oxygen inhibiting the dehydrogenase system. In any case the experimental results are in accord with the view of Libbrecht and Massart (1937) that in *in vitro* experiments oxygen at high pressure does not inactivate the dehydrogenase system in the presence of cyanide. Stadie, Riggs and Haugaard, (1944) have taken exception, however, to Libbrecht's and Massart's use of the term "l'oxygene actif" on the grounds that it is not sufficiently specific.

Although as previously demonstrated the cyanide-sensitive oxidase-cytochrome system is apparently not essential for the maintenance of pyloric sphincter tonus, the effects of NaCN and high oxygen pressure in combination justify the assumption that such enzyme system is present in this tissue and that in the tonus changes manifest, there is more than simply a failure of the oxygen to be made toxic by its "activation" through a cyanide-sensitive system.

The increased tonus of the cyanide treated tissue induced by the oxygen is not easily explained, but the finding that it is more pronounced at pressures of seventy-five pounds than it is with pressures of thirty pounds suggests that the concentration of the oxygen is somehow involved. The dehydrogenase system in this pyloric tissue is apparently capable of functioning in the absence of a cyanide-sensitive system, so this tonus increase may merely represent a greatly improved oxygen supply. This would imply either that the molecular oxygen in high concentration is itself available as a hydrogen acceptor or that there is some cyanide-refractory enzyme present (e.g., a cysteine system) which is capable of "activating" this oxygen in quantities sufficient to serve as a hydrogen acceptor but insufficient to cause a rapid poisoning of the dehydrogenase system.

If, however, as is maintained by Dixon and Elliott (1929) and Dale (1937) but vigorously denied by Warburg (1931) and his collaborators, the uptake of oxygen by tissue is not completely abolished by cyanide, there is the possibility that the temporary increase in tonus induced in our cyanide-treated pyloric tissue might have resulted from a combination of this residual ability of oxygen utilization and the increased oxygen pressure.

The fact that the pyloric sphincter tonus which has been elevated to a higher plateau by the high concentration of oxygen in the presence of cyanide is not maintained at this elevation, but shortly falls back to, and even below, the pre-compression level (curve B, fig. 3) might be construed as evidence that the high oxygen pressure had functionally removed the NaCN by oxidation. But such an interpretation is dismissed by the experimental finding that even after cyanide solution was exposed to and bubbled with oxygen at seventy-five pounds' pressure for an hour it still induced the characteristic depression of tonus when subsequently administered to longitudinal duodenal muscle at atmospheric pressure. Perhaps the most probable explanation for the failure of the pyloric sphincter

tonus to be maintained at the high plateau (curve *B*, fig. 3) would be that the dehydrogenase system is not permanently immune to the deleterious action of high oxygen pressure even when the cyanide-sensitive system is not functioning to produce "activated" oxygen.

In brief, the fall in tonus of pyloric sphincter muscle which is induced by its exposure to oxygen at high pressure can be delayed and even temporarily reversed by a previous administration of cyanide. It is suggested that this reversal results from a failure in the formation of "activated" oxygen consequent upon the poisoning of the oxidase-cytochrome system. Subtoxic amounts of activated oxygen made available through some non-cyanide-sensitive system and the high concentration of oxygen, result in a temporarily improved state and a rise in sphincter tonus which is later diminished when the amount of "activated" oxygen finally reaches a level such as to cause inactivation of the dehydrogenase. Reduction of the oxygen concentration by decompression to atmospheric pressure frees the dehydrogenase, at least partially, and as a result the sphincter tonus returns to normal (curve *B*, fig. 3).

Circular duodenal muscle. In this series, strips of circular duodenal muscle about 5 mm. wide, taken from the upper part of the duodenum were set up in the Tyrode solution bath as described for pyloric sphincter. Figure 4 illustrates the various tonus changes produced in this tissue by its exposure to high oxygen pressure and the administration of cyanide. In curve *A*, the effects of oxygen at seventy-five pounds' pressure alone are shown; compression (*C*) caused an abrupt fall in tonus beginning within five minutes of the end of compression. This initial fall occurred without exception in each of the seven experiments of this series. The similarity between this initial response of the circular duodenal muscle and that recorded from pyloric sphincter (curve *A*, fig. 1), together with the finding that this initial tonus drop may be prevented in both tissues by previous administration of cyanide (fig. 3*B*, 4*C*), supports the contention that the factors responsible for the tonus drop induced in both of these tissues by high oxygen pressure acting alone, are identical, viz., as suggested above in connection with the pyloric sphincter, the poisoning of the dehydrogenase system by the formation of unduly large amount of "activated" oxygen.

Aside, however, from the similarity of the initial drop in tonus and the reaction to high oxygen pressure after cyanide administration, the circular duodenal muscle differs markedly from the pyloric sphincter in its subsequent reaction to the high oxygen pressure. In the circular duodenal muscle the initially depressed tonus does not persist throughout the period of the maintained O_2 pressure; it recovers, so that the tonus may temporarily supersede the precompression level. Take for example figure 3*A*, here after about fifteen minutes of exposure to the increased pressure, the oxygen, even though it was present in high concentration would appear to be temporarily innocuous, a condition somewhat similar to that seen when the tissue has been previously treated with cyanide. In each of the experiments this recovery of tonus, which appeared within seventeen minutes after compression, might be interpreted as arising from a parallel recovery of the dehydrogenase system which was initially inhibited by the high oxygen pressure.

It is very unlikely that such a recovery could be a rapidly-acquired tolerance of the enzyme for the high oxygen pressure. A more reasonable explanation would seem to lie in a reduction of the amount of "activated" oxygen to a level where it ceases to exert a toxic action on the dehydrogenase system which then again becomes functional. But how might a diminution of "activated" oxygen to subtoxic levels come about? Perhaps the best answer would be that it is a direct consequence of an impairment of that enzyme system responsible for the activation of oxygen, viz., the cyanide-sensitive oxidase-cytochrome system. Taken as a whole, then, the results indirectly suggest that not only the high-oxygen-sensitive dehydrogenase system, but also the cyanide-sensitive oxidase-cytochrome system of the circular duodenal muscle is inhibited or damaged by oxygen at high pressure.

Further support for this view was derived from a study of the effect of high oxygen pressure on circular duodenal muscle after its cytochrome system had been specifically inhibited by cyanide. As shown in curve *B*, figure 4, cyanide of itself, administered in a final concentration of 1:15,000 failed to alter the tonus. The effect of superimposing oxygen at seventy-five pounds' pressure on such a cyanide-treated muscle (a fresh strip of circular duodenal muscle from the same animal as used in *A* and *B* of fig. 4) is shown in curve *C*, figure 4; administration of NaCN to a final concentration of 1:15,000 to poison the cytochrome system was without effect on the tonus, but now a subsequent exposure to oxygen at seventy-five pounds' pressure caused an increase in tonus instead of an initial fall, as had occurred in curve *A*. Thus the immediate effect of oxygen at high pressure on the tonus of the tissue previously treated with cyanide is similar to its delayed effect (after 15 min. exposure) on the untreated muscle. This similarity suggests that the fifteen minutes' exposure to high oxygen pressure by itself, as well as cyanide by itself, inhibits the oxygen activating enzyme and so permits recovery, at least temporarily, of the dehydrogenase system.

The post NaCN rise in tonus of circular duodenal muscle induced by the high oxygen, like that seen in the pyloric sphincter muscle under the same conditions (curve *B*, fig. 3) may possibly be attributed to an improved oxygen supply through the non-cyanide-sensitive systems. The failure to maintain the newly attained elevation of tonus (fig. 3*B*, 4*A*, 4*C*) is another feature common to the curves of both pyloric sphincter and duodenal circular muscle and perhaps is explainable on the basis of a temporarily improved state which persists only until the "activated" oxygen made available by the non-cyanide sensitive system has attained a level at which it becomes toxic.

The only significant difference, then, between the response of the duodenal circular muscle and that of the pyloric sphincter muscle to high oxygen pressure seen in these curves aside from a somewhat more precipitous initial fall in tonus in the duodenal tissue (fig. 3*A*, fig. 4*A*) is the pronounced secondary rise in duodenal tonus which appears within about fifteen minutes following the oxygen compression.

SUMMARY

Changes in tonus elicited from freshly isolated smooth muscles (rabbit) by oxygen at high barometric pressure and by NaCN were studied in an attempt to

explain the toxic action of high oxygen pressures on these tissues and their respiratory enzymes. The results indicate that pyloric sphincter tonus is sharply decreased by exposure to oxygen at thirty and seventy-five pounds' pressure, but is unaltered by NaCN in concentrations which inactivate the oxidase-cytochrome system. Since oxygen at high pressure is known to selectively inactivate the dehydrogenase system, it is concluded that this enzyme system is more important than the oxidase-cytochrome system to that metabolism upon which the maintenance of pyloric sphincter tonus is dependent.

Cyanide caused a rapid onset of a precipitous fall of tonus in freshly isolated longitudinal duodenal muscle but oxygen at high pressure reduced the tonus of fresh strips of this muscle only after a relatively prolonged exposure. It was concluded, therefore, that the oxidase-cytochrome system rather than the dehydrogenase system plays the more outstanding rôle in the control of the metabolism responsible for the maintenance of tonus in longitudinal duodenal muscle. It would appear then that the oxidative metabolism of the slowly acting pyloric sphincter tissue is governed largely by the relatively slow dehydrogenase enzyme system while that of the more active longitudinal duodenal muscle is controlled predominantly by the oxidase-cytochrome system which is known to be a more rapidly acting enzyme.

Oxygen at high pressure fails to cause a decrease in pyloric sphincter tonus if this muscle has been treated with NaCN prior to its exposure to the oxygen pressure. It would appear, therefore, that oxygen must be "activated" by a cyanide-sensitive system before it is capable of adversely affecting pyloric tonus and inhibiting the dehydrogenase system of this tissue.

Oxygen at high pressure induced an initial fall in tonus of circular duodenal muscle; with continued exposure there followed a secondary rise in tonus. Treatment of this tissue with NaCN prior to its exposure to high oxygen, however, completely eliminated the initial fall in tonus seen in the untreated tissue and instead there occurred an immediate rise in tonus. It was suggested that these effects might be interpreted as due to a poisoning of the oxygen-activating, cyanide-sensitive (oxidase-cytochrome) enzyme systems, the consequent decrease of "activated" oxygen to subtoxic levels and a resultant temporary resumption of function of the dehydrogenase system following an initial period of inhibition. This implies that the oxidase-cytochrome system, as well as the dehydrogenase system, is affected in oxygen poisoning.

It is suggested that the differences found in the responses of freshly isolated and two or three day old (kept in the cold room) longitudinal muscle to NaCN are indicative of a shift in the functioning of the respiratory enzyme systems following the tissue's isolation.

REFERENCES

- BEAN, J. W. AND D. F. BOHR. *This Journal* 130: 445, 1940.
BOHR, D. F. AND J. W. BEAN. *This Journal* 131: 338, 1940.
BRASSFIELD, C. R. AND R. GESELL. *Fed. Proc.* 1: 10, 1942.
CANNON, W. B. AND A. ROSENBLUETH. *Autonomic neuro-effector systems*. MacMillan Co., 1937.
CLARK, A. J. *Quart. J. Exper. Physiol.* 25: 181, 1935.

- DALE, A. S. J. Physiol. 89: 316, 1937.
DIXON, M. AND K. A. C. ELLIOTT. Biochem. J. 23: 812, 1929.
EVANS, C. L. J. Physiol. 53: 17, 1919.
GARRY, R. C. J. Physiol. 66: 235, 1923.
GESELL, R., C. R. BRASSFIELD AND M. A. HAMILTON. This Journal 136: 604, 1942.
HADIDIAN, Z. AND H. HOAGLAND. J. Gen. Physiol. 23: 81, 1939.
HOPKINS, F. O. AND E. J. MORGAN. Biochem. J. 32: 611, 1938.
JOHNSON, J. M., W. T. McCLOSKEY AND C. VOEGTLIN. This Journal 83: 15, 1927.
LIBBRECHT, W. AND L. MASSART. Compt. rend. Soc. Biol. 124: 299, 1937.
RAPER, H. S. Advancement of Science 2: 217, 1940.
STADIE, W. C., B. C. RIGGS AND N. HAUGAARD. Am. J. Med. Sc. 207: 84, 1944.
WARBURG, O. Biochem. J. 231: 493, 1931.

THE INNERVATION OF THE INTEROSSEOUS MUSCLES AND THE MECHANISM OF THE "TOE SPREADING REFLEX" OF THE HIND LIMB IN THE CAT

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Since the extensive investigations of Ruge (1878) and Cunningham (1882) it generally has been accepted that the interosseous muscles of the mammalian pes are innervated by the lateral plantar nerve, a terminal branch of the tibial nerve. In a recent paper Altschul and Turner (1942) claimed, however, that in the cat and the rabbit the dorsal interossei of the hind limb are supplied with motor fibers exclusively by the common peroneal nerve. Were this true, it would be an outstanding and important exception to the concept of nerve-muscle specificity. It therefore requires confirmation.

Altschul and Turner have based their conclusions entirely upon physiological evidence. In interpreting this evidence they have made two major assumptions. First, they have assumed that the interosseous muscles of the cat are identical with those of man in both form and action, and hence the active agents in the "toe spreading reflex" of this animal. In this reflex, the toes are extended and widely spread and the claws protruded or unsheathed when the animal is raised vertically from the ground. Second, since stimulation of the common peroneal nerve—and of this nerve alone—produces this action, they have assumed that the interosseous muscles of the cat are innervated by motor fibers of this nerve. They seem not to have considered the possibility that muscles other than the interossei might be producing the reaction in question. Apparently they have not studied the morphology nor determined the specific actions of the various pedal muscles in either cat or rabbit, but have taken it for granted that they are identical with those of man. We have studied these muscles and their innervations in seven cats, including careful and thorough electrical stimulation of muscles and nerves in both hind limbs of two animals anesthetized by intraperitoneal injection of nembutal.

ANATOMICAL FINDINGS. The arrangements of the pedal muscles of the cat, especially the intrinsic, differ so markedly from those of man, and are so important in an understanding of their actions, that they bear some detailed description. Deep to the long flexor tendons of the toes there are, as in other mammals, two layers of plantar muscles, separated by the deep division of the lateral plantar nerve and its branches (see fig. 1). The more superficial (plantar) of these is the layer of adductors (or contrahentes), comprising three small muscles—*m. adductor indicis* (described as a part of the interosseous muscle of the second toe by both Reighard and Jennings, 1938, and Davison, 1937; not recognized by Mivart, 1892), inserted upon the fibular base of the second toe; *m. adductor digiti quinti* ("*m. adductor medius digiti quinti*" of both Reighard and Jennings and Davison;

not described by Mivart), inserted upon the tibial base of the fifth toe; and *m. adductor-opponens digiti quinti* ("*m. opponens minimi sive quinti digiti*" of Reighard and Jennings, Davison, Mivart and Jayne), inserted upon the tibial aspect of the shaft of metatarsale V.

The deeper, heavier layer comprises the interosseous muscles, covering the plantar aspects of the metatarsal bones. These, however, are not so truly interosseous in position as they are in man and some other mammals, for the metatarsal bones of the cat are so closely adpressed that they are confined to the sole of the foot. They therefore are not really differentiated into dorsal and plantar sets as in man, as long ago shown by McMurrich (1907). Instead, they are true *musculi flexores breves profundi* as found in numerous mammals and other tetrapods. The two *musculi interossei* of each digit arise in common from the plantar base of their respective metatarsal bone and the adjacent tarsus. They eventually diverge to separate insertions on the tibial and fibular sides of the digit. Each interosseous muscle (save only the fibular component of digit V) consists of two distinct parts: 1. A heavy, dorsal portion, largely fleshy, which

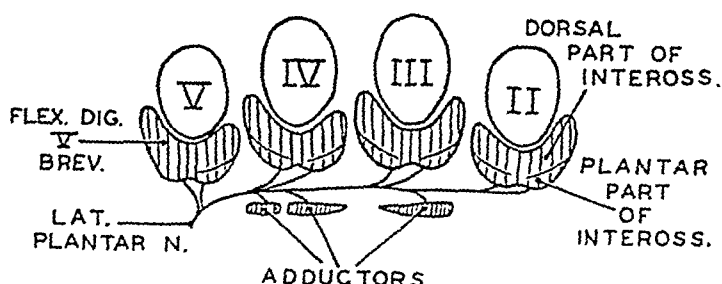


Fig. 1. Diagrammatic cross-section of the foot of the cat, through the proximal metatarsus, to show the interosseous and adductor muscles. The course of the deep branch of the lateral plantar nerve is indicated. The adductor muscles, from left to right, are *m. adductor-opponens digiti quinti*, *m. adductor digiti quinti* and *m. adductor indicis*.

is inserted into the plantar base of the proximal phalanx (including the sesamoid bone); usually, the more lateral part forms a distinct fascicle that is inserted by a slender tendon. 2. The smaller, plantar portion forms a long, strong tendon that passes around the side of the metatarso-phalangeal joint to attain the dorsal surface of the digit. Here it not only joins the corresponding tendon of the interosseous muscle of the opposite side of the digit, but also the tendons of *mm. extensor digitorum longus* and *extensor digitorum brevis* (figs. 2 and 3). The four form a common tendon which is strongly attached to the base of the middle phalanx and then continued forward to be inserted upon the terminal phalanx (see fig. 3). We have been unable to separate this common tendon into definite components such as Willan (1912) found in man. The fibular interosseous muscle of digit V (more properly termed *m. flexor digiti quinti brevis*, but not "abductor brevis" as by Reighard and Jennings and Davison) differs in that it sends no tendon to the dorsum of the digit, but is inserted wholly into the base of the proximal phalanx. The common dorsal tendon of toe V thus is formed only by the tendons of *musculi interossei* (tibialis), *extensor digitorum longus*

and peroneus digiti quinti. The adductor and interosseous muscles clearly are innervated by branches of the lateral plantar nerve. Thus it is evident, from purely morphological grounds, that the interosseous muscles of the cat have different actions than in man. Furthermore, to speak of "dorsal interossei," as opposed to "plantar interossei," in the cat is entirely gratuitous.

The muscles of the dorsum of the foot, all innervated by branches of the common peroneal nerve, merit careful attention (see fig. 2). *M. extensor digitorum longus* arises from the lateral condyle of the femur. Distally, it forms a tendon for each of the four toes. These join the respective common dorsal tendons as described above.

M. extensor digitorum brevis is a powerful muscle composed of three bellies. Origin is from the fibular side of the tarsus (especially calcaneus), with accessory slips from the metatarsal bases and adjacent distal tarsus. The tendon of the tibial belly divides into approximately equal branches; one of these becomes a part of the common dorsal tendon of toe II, while the other is attached to metatarso-phalangeal joint-capsule III (there may be a slender accessory tendon to metatarso-phalangeal joint-capsule II). The tendon of the middle belly also splits into two parts—the stronger of these passes along the fibular side of the third metatarsal bone, is strongly attached to the base of the proximal phalanx by a tendinous expansion, and continues forward to join the dorsal tendon of the fibular interosseous muscle of toe III, and, through this, the common tendon; the weaker branch of the tendon is inserted dorsally into metatarso-phalangeal joint-capsule IV. The tendon of the fibular belly continues without division along the fibular border of toe IV, being inserted in the manner of the stronger branch of the tendon of the middle belly.

M. peroneus digiti quinti (incorrectly termed "*m. peroneus tertius*" by Reighard and Jennings, Davison, and Jayne) arises from the fibula in association with the other peroneal muscles. Its long, slender tendon passes along the outer margin of the foot to its insertions on the fibular base of the proximal phalanx and the common tendon of digit V.

EXPERIMENTAL FINDINGS. In our stimulation experiments we used a sine wave current of 60 cycles applied through bipolar electrodes.

As Altschul and Turner have stated, stimulation of the common peroneal nerve causes pronounced spreading of the digits and unsheathing of the claws. In our animals, the foot also was dorsoflexed and all four toes were extended. The digital spreading was most pronounced in toes IV and (especially) V. It also was found, in agreement with the above authors, that stimulation of the tibial nerve does not produce this action. We have noted that faradization of this nerve in the crus (below the innervation of the long digital flexors) causes marked adduction of the toes, with accompanying flexion of the proximal and extension of the middle phalanges. This movement is largely, if not entirely, due to the action of the interosseous muscles; for when these muscles were exposed by careful reflection of the more superficial layers (so that only the interosseous and adductor muscles remained intact), and the tibial nerve or its distal stump was stimulated, the same action followed upon their contraction. Spreading of

the digits and protrusion of the claws did not take place. On the other hand, stimulation of the common peroneal nerve never caused any contraction of the exposed musculi interossei. Direct stimulation of these muscles reproduced the results obtained through faradization of the tibial nerve. It thus is evident that the interosseous muscles of the cat have no active part in the "toe spreading reflex", nor are they supplied with motor fibers by the common peroneal nerve. Rather, they are innervated by the tibial nerve as in other mammals.

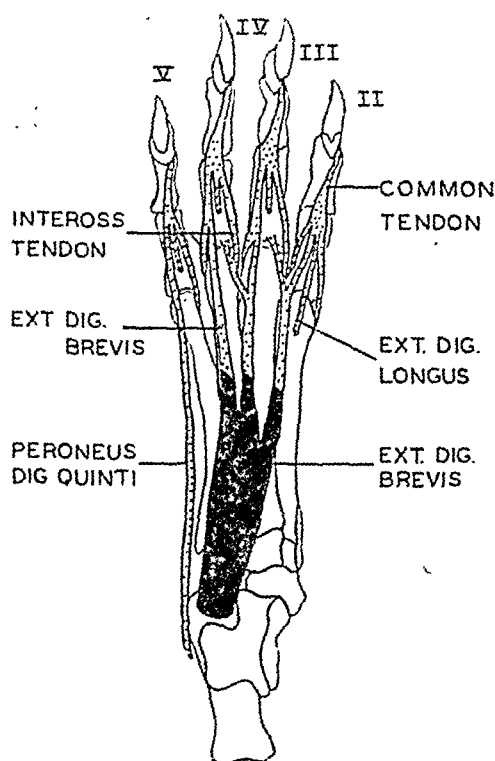


Fig. 2

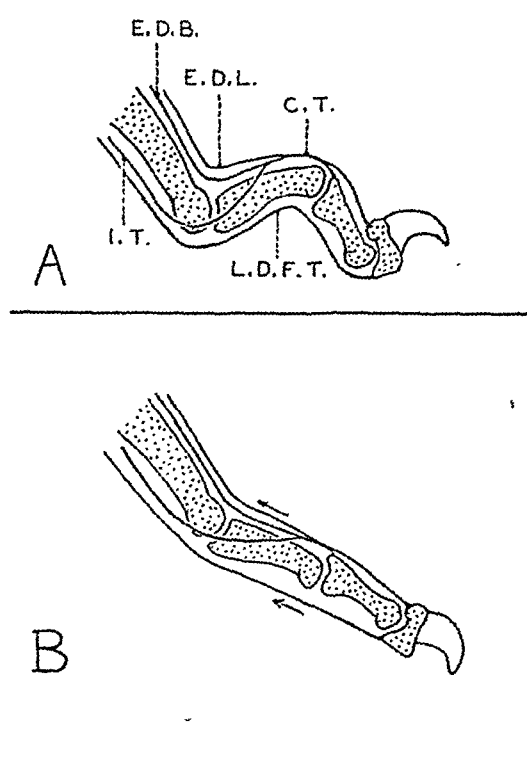


Fig. 3

Fig. 2. Dorsal view of the foot of the cat, to show the tendons of the digital extensors. The contributions of the interosseous muscles to the common dorsal tendons also are included. The outline of the skeleton is after Jayne.

Fig. 3. Medial views of the foot of the cat (showing the distal part of the metatarsus and the three phalanges), to illustrate the mechanism of the claw protrusion that accompanies the "toe spreading reflex". The tendons of the muscles responsible for this action are shown: *M. extensor digitorum longus* (*E. D. L.*), *m. extensor digitorum brevis* (*E. D. B.*) and the long digital flexors (*L. D. F.*). The contribution of the interosseous muscle (*I. T.*) to the common dorsal tendon (*C. T.*) also is indicated. A (above), foot at rest in the standing position, with claws retracted. B (below), foot with toes extended and claws protruded.

It has been clearly demonstrated in our experiments that the "toe spreading reflex" is effected entirely by the dorsal muscles previously described—*musculi extensor digitorum longus*, *extensor digitorum brevis* and *peroneus digiti quinti*. The "toe spreading" is largely, if not entirely, produced by the action of the powerful *m. extensor digitorum brevis* and *m. peroneus digiti quinti*. The rôle of *m. extensor digitorum longus* clearly is accessory in this movement;

for when its tendons are severed, and the other muscles left intact, spreading of the toes consequent to stimulation of the common peroneal nerve was not diminished perceptibly. But section of the tendons of *musculi extensor digitorum brevis* and *peroneus digiti quinti* did abolish this reaction, despite the integrity of *musculi interossei*.

The unsheathing of the claws, which is a part of the "toe spreading reflex" in the cat, is consequent to the digital extension. In the normal, standing position, with retracted or sheathed claws, the relations of metatarsals and phalanges are approximately as shown in figure 3A. When the middle phalanges are extended by *m. extensor digitorum brevis* (and *m. peroneus digiti quinti*), the tension thus applied to the tendons of the long digital flexors (which are inserted upon the terminal phalanges) causes the terminal phalanges to be flexed and the claws thus unsheathed (fig. 3B). Although the plantar portions of *musculi interossei* have dorsal insertions in common with *musculi extensor digitorum longus* and *extensor digitorum brevis*, they have no share in this action, apparently because of their unfavorable leverage.

The entire "toe spreading reflex" may be simulated in both living and dead animals by pulling the dorsal skin of the pes in a proximal direction.

We have not studied the rabbit, in which, according to Altschul and Turner, the same conditions (excepting claw unsheathing) prevail as in the cat. In view, however, of their evident misinterpretation respecting the latter animal, this has not been deemed necessary.

SUMMARY

1. The pedal interosseous muscles of the cat receive their motor innervation from the tibial nerve, and not from the common peroneal nerve as claimed by Altschul and Turner.
2. These muscles, in contrast with those of man, play no active part in spreading of the toes. Their action is, rather, to approximate the digits with flexion of the proximal and extension of the middle phalanges.
3. The spreading of the toes and unsheathing of the claws is produced by muscles innervated by the common peroneal nerve, especially *musculi extensor digitorum brevis* and *peroneus digiti quinti*.

REFERENCES

- ALTSCHUL, R. AND K. P. TURNER. *This Journal* 137: 247, 1942.
- CUNNINGHAM, D. J. Report on the Marsupialia. *Challenger Reports, Zoology* 5: pt. 16, 192 pp., 1882.
- DAVISON, A. *Mammalian anatomy with special reference to the cat*. 6th ed., Blakiston, Philadelphia, 1937.
- JAYNE, H. *Mammalian anatomy*. Lippincott, Philadelphia, 1893.
- McMURRICH, J. P. *Am. J. Anat.* 6: 407, 1907.
- MIVART, ST. G. *The cat*. Scribner, New York, 1892.
- REIGHARD, J. AND H. S. JENNINGS. *Anatomy of the cat*. 3rd ed., Holt, New York, 1938.
- RUGE, G. *Morph. Jahrb.* 4: 644, 1878.
- WILLAN, R. *Anat. Anz.* 42: 145, 1912.

THE OXYGEN CONSUMPTION OF EYE MUSCLES OF THYROIDECTOMIZED AND THYROXIN- INJECTED GUINEA PIGS¹

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The function of the extraocular muscles is known to be profoundly affected by disturbances of the thyroid gland. Weakness of the eye muscles, causing diplopia, and sometimes an almost complete paralysis of one or more of them, often attends hyperthyroidism. Exophthalmos which, in part at least, is dependent on alteration of thyroid function, has been characterized in one group of cases by the enlargement and pathological modification of the extraocular muscles (1). The fact that oxygen consumption of dystrophic skeletal muscles, caused by dietary deficiencies, is affected (2, 3, 4), suggests that the metabolic rate of the eye muscles of hypo- and hyperthyroid animals might indicate a basis for the frequent alterations which are associated with variations in the thyroid state and exophthalmos. The eye muscles seem to be particularly suited to tissue respiration studies, since their small size makes it possible to study the metabolism of a practically intact muscle. The simultaneous determination of the oxygen uptake of the extra-ocular and other muscles of the same animals permits a comparison of the quantitative effect of the thyroid hormone on the oxidative metabolism of different muscles.

EXPERIMENTAL. Besides the eye muscles, the latissimus dorsi and diaphragm were chosen for this study, since they represent quite different structural and functional types of muscle tissue. The extra-ocular muscles differ from other skeletal muscles in the amount of sarcoplasm, in the position of nuclei, some of which are centrally located, and in the coarseness of striations and the small size of the fibers. The ratio of nerve to muscle fibers is very high, which may be correlated with the extreme activity of these muscles and their ability to make very precise movements. The latissimus dorsi is a much more typical skeletal muscle both in structure and in function. It is adaptable to metabolic studies because of its thinness. Diaphragm was selected because its regular and continuous activity make it more comparable to the eye muscles, in this respect. Its thickness adapts the diaphragm to studies on respiration; furthermore, many investigations of muscle metabolism have been conducted on it so that its behavior is well known.

Oxygen consumption of the tissues was determined in a Warburg apparatus equipped with 10 ml. flasks. Each experiment consisted of paired determinations of the oxygen uptake of each of the three muscle types from one animal. Fifty

¹ Aided by a grant from the Francis I. and Elizabeth C. Proctor Fund. The work on this paper was done at the Marine Biological Laboratory, Woods Hole, Mass.

to 75 mgm. of tissue were placed in 2 cc. of a salt solution made by mixing 2 cc. of 1.15 per cent KCl, 2 cc. of 1.22 per cent CaCl_2 and 100 cc. of 0.9 per cent NaCl brought to pH 7.4 by the addition of 10 cc. to 20 cc. phosphate buffer. Two hundred milligrams of glucose were added to 100 cc. of the above stock solution. All experiments were conducted in an atmosphere of oxygen at $37.5^\circ \text{C.} \pm 0.5^\circ$.

Twenty-one guinea pigs, six normal, six thyroidectomized, and nine injected with thyroxin, were used in this study. Each thyroidectomized animal was a littermate of one of the normal control animals. Some of the hyperthyroid guinea pigs had littermates in the normal and thyroidectomized groups. Animals of all groups were autopsied at ages ranging from 72 to 122 days. The ages of the paired normal and thyroidectomized animals did not differ more than three days. The guinea pigs were thyroidectomized when from 8 to 18 days of age, and the completeness of the thyroid ablation was checked at autopsy by careful search for remnants with a binocular dissecting microscope. The few incompletely thyroidectomized animals were not included in the data presented. Length of the post-operative period varied from 57 to 67 days. Nine intact guinea pigs were injected with thyroxin (0.1 mgm. per day) for the 9 to 12 days preceding autopsy. Subcutaneous injections were made on the abdomen, far from the region covered by the latissimus dorsi. The body weights of the normal, thyroxin injected, and thyroidectomized guinea pigs averaged 433, 365 and 294 grams respectively and there was very little variation in these groups.

The extra-ocular muscles (excepting the levator) of the right and left eyes were placed in separate flasks. The two largest muscles were carefully split to increase the exposed surface. The two specimens of diaphragm were from the right and left sides and from the most dorsal and thinnest portions. The two specimens of the latissimus dorsi were prepared by splitting the muscles in such a way that a minimal number of fibers were injured. Very little tissue damage was done, particularly in the eye and diaphragm preparations. In each of these, the muscles were cut at their point of origin, the tendon end remaining intact. The muscles were weighed rapidly on a torsion balance and placed in prepared respirometer flasks. The preparations were set up quickly, and the order in which the muscles were placed in the flasks was varied, so that the time factor was not greater in one than in another.² The thickness of the pieces of muscle tissue was determined, and the availability of oxygen to the cells in the central region of the muscle strips was calculated according to Krogh's formula (5). It was found that all preparations were sufficiently thin for adequate oxygen diffusion. The respiration rate of the muscle samples was determined over a three-hour period, at the end of which the pH of the medium was tested, and the pieces of tissue placed on small, weighed pieces of coverslips. The tissue was dried over night in an oven at 100°C , cooled in a desiccator, and weighed on a torsion balance, to determine the solid content. The oxygen consumption per hour was calculated in terms of cubic millimeters of oxygen per milligram of tissue, both fresh and dry weight. The technique was checked by determinations of the oxygen consumption of normal rat diaphragms. Values obtained were found to agree very well with those cited in the literature.

² My thanks are due Elizabeth Smelser for her assistance in this work.

RESULTS. It was apparent after the first few experiments, that the oxygen consumption per unit weight of tissue was quite different in the three muscles studied, and that the oxygen requirement of normal eye muscles, *in vitro*, was very high. The QO_2 (cu.mm. O_2 per mgm. of dry muscle per hr.) of the normal eye muscle was 87.5 per cent higher than that of diaphragm, and 187.5 per cent

TABLE 1

The oxygen consumption of muscles of normal, thyroidectomized and thyroxin injected guinea pigs

DIAPHRAGM			LATISSIMUS DORSI			EXTRAOCULAR		
Normal	Thyroid-ectomized	Thyroxin injected	Normal	Thyroid-ectomized	Thyroxin injected	Normal	Thyroid-ectomized	Thyroxin injected
A. cu. mm. O_2 /mgm. dry wt./hour								
4.5	2.9	5.8	3.1	2.5	3.3	8.0	7.4	7.8
3.5	3.1	4.9	2.0	2.5	3.2	7.1	7.7	7.1
4.0	2.7	6.8	2.7	2.8	3.1	7.6	6.4	8.8
4.0	2.7	7.9	3.1	2.2	3.9	7.9	6.5	9.3
4.5	3.0	6.0	2.7	1.7	3.5	7.8	7.1	7.5
4.4	3.3	7.4	2.5	3.3	4.0	8.0	8.2	9.0
		4.9			3.2			8.6
		6.5			3.3			8.7
		6.8			2.4			10.4
Mean:	4.15	2.95	6.3	2.7	3.3	7.7	7.2	8.6
ϵ_M :	0.16	0.10	0.34	0.17	0.23	0.15	0.30	0.34
B. cu. mm. O_2 /mgm. fresh wt./hour								
0.63	0.45	1.00	0.42	0.27	0.53	0.92	0.83	1.13
0.57	0.46	0.95	0.28	0.23	0.56	0.97	0.88	1.03
0.62	0.41	1.09	0.39	0.30	0.36	1.02	0.72	1.10
0.59	0.37	1.35	0.35	0.23	0.48	0.95	0.75	1.27
0.79	0.47	0.93	0.41	0.23	0.51	0.94	0.84	1.19
0.67	0.47	1.40	0.31	0.22	0.61	1.11	0.90	1.40
		0.88			0.46			1.08
		0.95			0.56			1.14
		1.03			0.31			1.28
Mean:	0.64	0.44	1.06	0.36	0.25	0.49	0.82	1.18
ϵ_M :	0.03	0.014	0.06	0.02	0.01	0.03	0.028	0.04

$$\epsilon_M = \sqrt{\frac{\sum d^2}{n(n-1)}}$$

greater than that of the latissimus dorsi muscle of the same animals (table 1). These differences were consistent, and there was no overlapping of the values obtained for the three muscles.

Injection of thyroxin affected the QO_2 of all three muscles, but not to the same degree. Oxygen consumption of the diaphragm was increased greatly (53 per

cent), whereas that of the eye muscles was much less affected (11 per cent). Physical conditions did not prevent a greater increase in oxygen consumption of the eye muscles, for even at this higher rate of oxygen use the rate of diffusion of gases through the tissue was adequate. In addition, the eye muscles of the hyperthyroid animals were 16 per cent less in weight than in the normal animals. The increase in QO_2 of the latissimus dorsi was intermediate (24 per cent) between that of the diaphragm and eye muscles.

Thyroid ablation led to a marked but differential decrease in the QO_2 of all muscles. Again, the diaphragm was the most markedly affected, showing a decrease in oxygen consumption of 32 per cent based on the fresh tissue. The metabolism of the eye muscles of the thyroidectomized guinea pigs decreased slightly (17 per cent) in terms of the fresh tissue weight but showed only an insignificant change on the basis of the dry tissue weight. A very definite and statistically valid decrease in oxygen consumption of the latissimus muscle of the thyroidectomized animals occurred (32 per cent) in terms of fresh tissue weight. However, thyroid ablation also led to such a marked increase in the water con-

TABLE 2

The average solids per 100 mgm. fresh weight of muscle from normal, thyroidectomized and thyroxin injected guinea pigs

MUSCLE	THYROIDECTOMIZED 12 SPECIMENS		NORMAL 12 SPECIMENS		THYROXIN INJECTED 18 SPECIMENS	
	mgm.	%	mgm.	%	mgm.	%
Diaphragm.....	15.1	0.4	15.9	0.43	17.0	0.41
Latissimus dorsi.....	10.3	0.67	13.5	0.6	14.4	0.6
Extra-ocular.....	11.5	0.15	12.6	0.25	14.2	0.32

tent of this muscle that the solid constituent decreased 24 per cent, consequently the oxygen used per milligram of tissue solids was not significantly decreased.

The several muscles thus vary in their metabolic rate, and in the effect of the thyroid on this rate. They also differ in water content and in the effect that variations in the amount of thyroid hormone have on the tissue water balance.

The change in tissue solids of these muscles resulting from thyroxin injection and thyroid ablation is given in table 2. The diaphragm, the QO_2 of which was the most markedly affected by changes in the available thyroid hormone, was quite stable relative to its water content. The percentage of solid material found in the diaphragm was not significantly different from normal in the thyroidectomized or hyperthyroid groups. The solid content of the eye muscles, however, was decreased in the thyroidectomized (9 per cent), and increased (13 per cent) in the hyperthyroid animals. The thyroxin injections failed significantly to change the proportion of solids contained in the latissimus dorsi, but thyroid ablation caused a greater decrease (24 per cent) in such solids than was found in the eye muscles. That the guinea pigs in these experiments were thyroidectomized when very young may in part account for the marked effect on the water/solids ratio in these tissues. Thyroid ablation in adult animals in other experi-

ments did not result in as great an increase in the water content of the eye muscles (6).

DISCUSSION. The data reported here show that the eye muscles have an exceedingly high respiration rate even approaching that of some epithelial tissues, such as rabbit pancreas or rat placenta (7, 8). The QO_2 of these muscles may be correlated with their activity and the precision of their movements. A very high QO_2 was found in another active muscle, the laryngeal, by Alwall (9).

However, the variable degree in which the thyroid hormone affects the metabolism of different muscles seems of great significance. The present data are in substantial agreement with the reports of Foster (10), Dye (11) and Davis (12), who showed that thyroid ablation and thyroid administration affected the QO_2 of muscle tissue. Although the injection of thyroxin increases the oxygen consumption of muscle tissue the increase is not uniform in all muscles. Apparently that portion of the metabolic system which is acted upon by the thyroid hormone varies quantitatively in different muscles. In the eye muscles it seems to form a very small proportion of the total, as represented by oxygen consumption. This is shown in both experiments after thyroid ablation and thyroxin administration. The effect of the thyroid hormone on the metabolic rate of different muscles bears no relation to the activity or the normal rate of metabolism of this tissue. In these experiments the diaphragm was the most sensitive to the deprivation or administration of thyroid but was intermediate in its normal oxygen consumption and probably in activity also.

The present data are capable of quite different interpretations, depending on whether the results are based upon the fresh or dry weight of the tissues. It is difficult to determine whether the QO_2 based on dry or fresh weight of the latissimus dorsi and extraocular muscles represents more exactly the true oxygen consumption of these tissues in the thyroidectomized animals. If the increase in water were largely in the form of an edema it could be considered inert, metabolically, and the situation would be somewhat comparable to the fatty infiltration of the liver (13) in which the proportion of liver cells was simply reduced. No histological examinations have been made of the latissimus muscles, but a small amount of stainable interstitial fluid occurs in the eye muscles of hypothyroid guinea pigs (14).

Since variations in amount of thyroid hormone affected the QO_2 of the eye muscles so slightly, in comparison with the diaphragm, it seems that the changes in these muscles, which so frequently occur in thyroid disease, are not due directly to the amount of thyroid hormone in the circulation although it is possible that the influence of this hormone on the water balance of different muscles may be a causative factor. As stated earlier the thyroid hormone had but little effect on the water content of the diaphragm, but had a pronounced effect on the eye muscles. In experimental exophthalmos, the major structural change observed in the eye muscles has been the abnormal amount of water between the muscle fibers (15).

The eye muscles possessed a higher metabolic rate, in all animals, than did the other muscles, but the difference was much less in the hyperthyroid ani-

mals. This was, of course, due to the great sensitivity of the diaphragm metabolism, and the lack of response of the eye muscle metabolic system to the thyroid hormone. Apparently the metabolism of muscle tissue, as expressed by oxygen consumption, is the summation of the function of enzyme systems, some of which are rendered more active or are increased in amount by the thyroid hormone. This thyroid sensitive component is probably present in all muscles, but is a very important one, quantitatively, in the diaphragm, and almost negligible in the metabolic system of the extraocular muscles.

CONCLUSIONS

The oxygen consumption of the extraocular muscles in normal guinea pigs is much higher than that of the other muscles studied. This difference is less in the muscles of thyroxin treated animals. The effect of the thyroid hormone on the water content of muscle tissue appears to vary in different muscles, and to be entirely separate from its effect on their metabolism. Although the thyroid hormone markedly increased oxygen consumption of the diaphragm, it has very little effect on that of eye muscle. This suggests that the enzyme systems which are the site of action of the thyroid hormone, are present in variable amounts, or activity, in different muscle tissues.

REFERENCES

- (1) NAFFZIGER, H. *Arch. Ophth.* 9: 1, 1933.
- (2) VICTOR, J. *This Journal* 108: 229, 1934.
- (3) MADSEN, L. L. *J. Nutrition* 11: 471, 1936.
- (4) FRIEDMAN, I. AND H. A. MATTILL. *This Journal* 131: 595, 1941.
- (5) DIXON, M. *Manometric methods*. Cambridge University Press, 1933.
- (6) SMELSER, G. K. *This Journal* 140: 308, 1943.
- (7) STARE, F. J. AND C. A. ELVEHJEM. *This Journal* 105: 655, 1933.
- (8) WARBURG, O. *Biochem. Ztschr.* 184: 484, 1927.
- (9) ALWALL, N. *Acta. Med. Scandinav.* 102: 258, 1939.
- (10) FOSTER, G. L. *Proc. Soc. Exper. Biol. and Med.* 24: 334, 1927.
- (11) DYE, J. A. *This Journal* 105: 110, 1933.
- (12) DAVIS, J. E. AND A. B. HASTINGS. *This Journal* 105: 110, 1933.
- (13) HASTINGS, A. B. *J. Biol. Chem.* 129: 295, 1939.
- (14) SMELSER, G. K. *Anat. Rec.* 85: 245, 1943.
- (15) SMELSER, G. K. *Am. J. Ophth.* 20: 1189, 1937.

THE EXPERIMENTAL PRODUCTION OF A HYPERCHROMIC ANEMIA IN DOGS WHICH IS RESPONSIVE TO ANTI-PERNICIOUS ANEMIA TREATMENT¹

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The author has reported recently (1) that the daily administration of choline chloride to normal dogs produced a significant depression of their red blood cell counts within 10 to 15 days, presumably by decreasing the rate of erythropoiesis.

This paper proposes to report an accentuation of this anemia by the administration of more than one dose of choline daily, and the response of this anemia to liver injection U.S.P. and stomach U.S.P.

PROCEDURE. Seven dogs were maintained on a diet of Purina Dog Chow and rolled oats, with water ad libitum. This diet is adequate in the sense that dogs remain healthy and maintain or gain weight, even when kept on it for many months. Two of our dogs have thrived on this diet in our laboratory for over two years.

Red blood cell counts and hemoglobin percentages (Hellige) were determined frequently, and total leukocyte counts, hematocrit readings (Van Allen) and reticulocyte percentages were determined at least occasionally. Blood samples for these determinations were drawn from the external saphenous veins of the dogs while they were lying upon a table, blindfolded, in an unexcited and fairly basal condition, at least 7 (and usually 11) hours after any previous administration of drugs or food.

To produce the anemia, four of the dogs were given one dose of choline hydrochloride² daily (10 mgm. per kgm) by stomach tube at the start of the experiment and for at least 25 days. Then after a definite anemia had been established, they were put on two daily doses, each of 10 mgm. per kgm. of body weight. After a lower red cell level had been reached on this regime, a third daily dose was added to each dog's program. The single doses were kept at constant magnitude (i.e., 10 mgm. per kgm.) and they were spaced about 6 or 7 hours apart during the day. Although the program was occasionally varied slightly, doses were given usually at 9 a.m., 4 p.m. and 11 p.m. daily, including Sundays and holidays. One other dog was "Accelerated" by being put on 2 daily doses of choline 7 days after the start of medication, and on 3 daily doses beginning on the 26th day of medication. Two other dogs, who were new and not well acclimated to this laboratory, were given 3 daily doses of choline from the outset of their experiments and continued thereon.

Liver injection, U.S.P., was administered to three dogs after their anemias had reached an apparent maximum. It consisted chiefly of the daily intra-

¹ Research paper no. 551, journal series, University of Arkansas.

² Part of the choline chloride used in these experiments was a gift from the Lederle Laboratories, New York, New York.

muscular injection of 2 cc. of a purified solution of liver containing 1 U.S.P. unit per cc.³ One dog, however, received 0.5 cc. of a solution containing 15 U.S.P. units per cc., daily for 7 days. Choline feeding was continued at the regular rate throughout the periods of liver administration.

Stomach U.S.P. (Ventriculin⁴) was fed to one dog in daily doses of 20 grams, in addition to choline, after his anemia had been established.

Atropine sulfate (0.5 mgm. per kgm.) was given in dilute solution by stomach tube to one anemic dog 3 times daily, in addition to the three daily doses of choline.

RESULTS. Figure 1 shows the red blood cell counts of 5 dogs during the development of their anemias by choline feeding. It will be seen that the erythrocyte numbers of these dogs were reduced finally by 30 to 43 per cent, due to choline administration. Hemoglobin percentages were reduced, *but not so greatly*, and indeed not proportionately. Table 1 shows the status of the color index at different levels of the red cell counts in 2 dogs. For the purpose of computing the color index, the author has arbitrarily placed the dog's normal red blood cell count at 6 million, and the normal value for 100 per cent hemoglobin at 13 grams per 100 cc. of blood (or 90 per cent on the Wintrobe scale). This was done after inspecting data in articles by Wintrobe (2) and Van Loon and Clark (3) and also data assembled by this author. This anemia may be classed as macrocytic in type, since we found relative volume indices of 1.18 and 1.45 in 2 dogs in which it was calculated. Total leukocyte counts did not change much although they were observed to be diminished by about 12 per cent in three dogs. Reticulocyte percentages during the anemia varied from about 0.05 per cent to 0.6 per cent.

Gastric juice containing free hydrochloric acid was obtained from two dogs after an alcohol test meal or histamine injection. In three others no significant amount of juice was obtained, but this may have been due to imperfect technique.

Figure 2 shows the response of the anemia to purified solution of liver, which was injected intramuscularly into 3 dogs (long dashes, fig. 2). Two of the animals received 2 units daily for about 27 days, and they responded by regenerating at least 85 per cent of their red blood cell deficits during this time in spite of continued choline feeding. Hemoglobin regeneration lagged behind the erythrocyte production. One dog (fig. 2) served as a control for 3 weeks, and was then given a potent liver solution (15 units per cc.) in the dosage of $7\frac{1}{2}$ units per day, intramuscularly. He responded with a rapid rise in red cell count within the 7 days during which he was observed. Reticulocyte percentages arose to only 1.5 to 2.3 in these dogs during liver therapy. The sixth day of therapy showed peak reticulocytosis.

One dog was given atropine sulfate 3 times daily in addition to regular doses of choline. As will be seen in figure 2 (short dashes), this animal showed a red blood cell count increase that very closely resembled those obtained in the liver treated dogs. Hemoglobin percentage increased, but not as greatly or as fast

³ "Campolon" was generously supplied by Winthrop Chemical Co., New York, New York.

⁴ Ventriculin was kindly supplied by Parke-Davis & Co., Detroit, Michigan.

as the red cell number. The reticulocyte percentage reached only 1.1 per cent on the 8th day of atropine administration.

Ventriculin was fed one anemic dog in a daily dose of 20 grams for 12 days. In this time his erythrocyte number and hemoglobin percentage returned to its normal value, in spite of continued choline administration. On the 7th day of therapy, his reticulocyte percentage was determined to be 2.4.

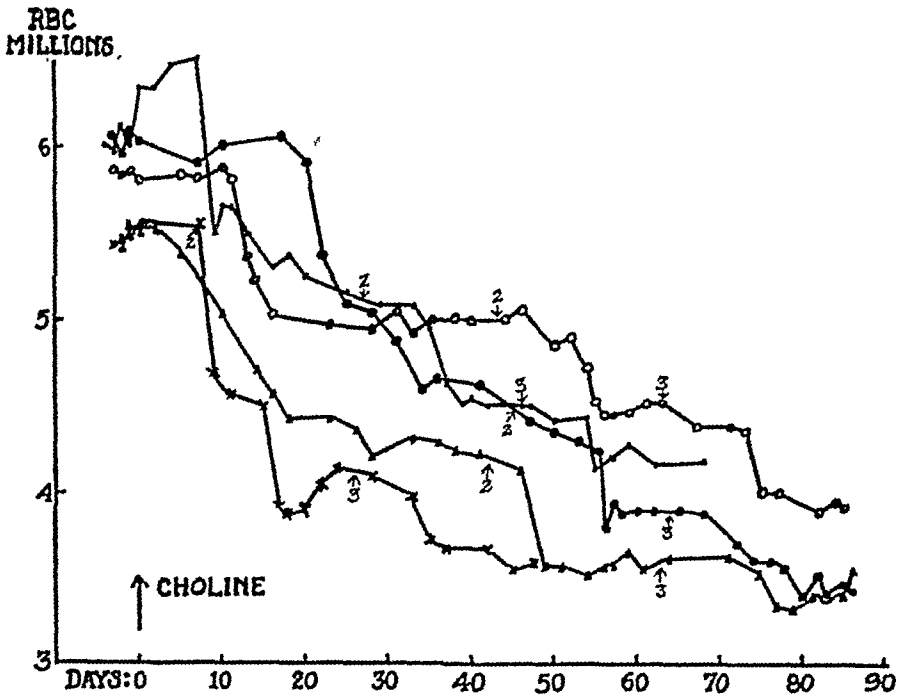


Fig. 1. Red blood cell counts obtained on five dogs during the development of choline anemia. 2 indicates commencement of the administration of two doses daily of choline. 3 indicates start of 3 doses daily for each dog as indicated.

TABLE 1

Color index changes in two anemic dogs treated by liver injection

	dog 4			dog 9		
	RBC millions	Hemoglobin per cent	C.I.	RBC millions	Hemoglobin per cent	C.I.
Normal control values	6.04	90	1.01	5.50	85	1.03
During anemia	3.51	64	1.22	3.50	53	1.10
During liver therapy	4.50	72	1.06	4.45	64	0.96
	5.10	75	0.98	4.75	66	0.92
	5.50	80	0.97	5.00	68	0.91

DISCUSSION. It appears in figure 1 that the use of more than one dose of choline daily augments the anemia that is produced by one dose daily. We have taken one anemic dog which had been receiving 3 daily doses of 100 mgm. each, and put him on a schedule in which he received only one large dose of 600 mgm. daily (not shown). He responded with a rise in his red cell count after about one week. Furthermore we have previously found that doubling the single daily

dose of choline did not increase its action in reducing experimental polycythemia. These facts lead us to believe that the magnitude of the response to choline is a function of the time of exposure to its action.

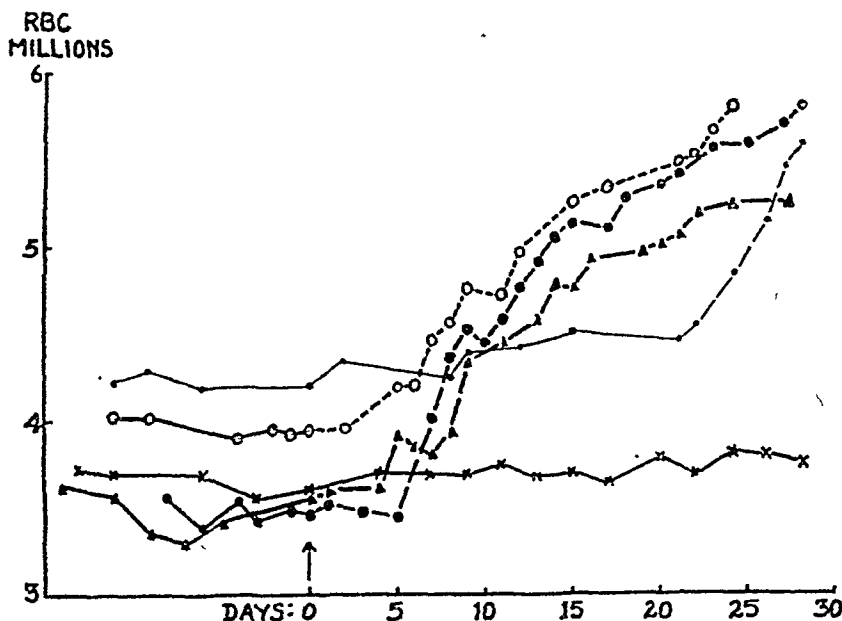


Fig. 2. The effect of liver injection or atropine upon anemic dogs. Long dashes indicate periods of liver administration in 3 dogs. Short dashes indicate simultaneous administration of atropine sulfate with choline in one dog.

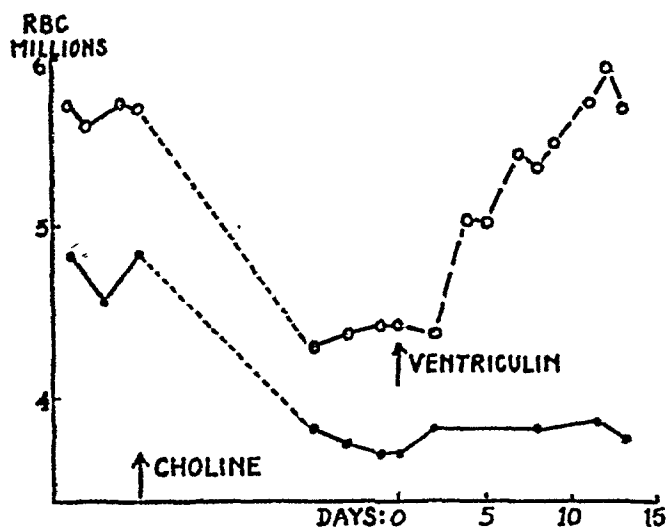


Fig. 3. The effect of stomach U.S.P. on the erythrocyte number of one anemic dog.

We believe, as we have stated previously (1, 4), that choline depresses erythropoiesis by increasing the blood and oxygen supply to bone marrow, through its vasodilator action. Since atropine antagonises the action of choline in these experiments (fig. 2) and in experiments reported previously (4) the action of the latter must be a muscarinic action. We believe that the important action must be *vasodilatation*, especially in view of our previous experiments (4, 5) in which

other vasodilator drugs such as nitrites, aminophylline and certain choline esters and ethers were shown to be effective in reducing experimental polycythemias.

We cannot say that it is the anti-pernicious anemia principle that causes the regeneration of red blood cells in these experiments, although such a conclusion is suggested by the fact that two brands of purified solution of liver, as well as ventriculin, were effective in returning the erythrocyte numbers virtually to normal.

We do not know the mechanism by which liver and stomach, U.S.P., cause a remission of this "choline anemia." The remission resembles superficially, at least, that induced by atropine in one dog of our series. In acute experiments we have been unable to find evidence for any atropine-like action or any circulatory action in highly potent purified solutions of liver. We were unable to find any evidence for the presence of choline oxidase in the liver solutions, as was perhaps to be expected since this enzyme is not very extractable from liver (6). It seems quite possible to the author that liver injection may contain or form a synthesizing enzyme which removes free choline.

The possibility exists that dogs with choline-induced anemia could be used for the biological assay of anti-pernicious anemia preparations.

It should be pointed out, however, that great care must be taken to draw blood samples only when the animals are unexcited and in a resting state, if experiments of this sort are to be successfully conducted.

CONCLUSIONS

The oral administration of 3 doses daily of choline chloride (10 mgm. per kgm. each) to 5 dogs produced reductions of from 30 to 43 per cent in their red blood cell counts. Milder anemias were produced in 2 other animals. The anemias, so produced, are hyperchromic and probably are the result of a depression of erythropoiesis.

The intramuscular injection of adequate doses of purified solutions of liver into 3 anemic dogs caused their erythrocyte numbers to respond with increases which were marked by the 7th day, and (in two animals) approximated normal after 4 weeks. These responses occurred in spite of continued choline administration.

Stomach, U.S.P., fed in daily doses of 20 grams to one mildly anemic dog, caused a return of his red cell number and hemoglobin percentage to normal within 12 days, in spite of continued choline feeding.

The oral administration of atropine sulfate 3 times daily to one anemic dog caused his red cell count to return to normal in 4 weeks in spite of continued choline feeding.

REFERENCES

- (1) DAVIS, J. E. This Journal (in press).
- (2) WINTROBE, M. M., H. B. SHUMACKER AND W. J. SCHMIDT. This Journal 114: 502, 1936.
- (3) VAN LOON, E., JR. AND B. B. CLARK. J. Lab. and Clin. Med. 28: 1575, 1943.
- (4) DAVIS, J. E. J. Pharmacol. and Exper. Therap. 70: 403, 1940.
- (5) DAVIS, J. E. J. Pharmacol. and Exper. Therap. 73: 162, 1941.
- (6) BERNHEIM, F. AND M. L. C. BERNHEIM. This Journal 121: 55, 1938.

THE RATE OF TRANSCAPILLARY EXCHANGE OF SODIUM IN NORMAL AND SHOCKED DOGS¹

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A generalized increase in "capillary permeability" is considered a critical factor in those theories of traumatic shock which picture as a vicious cycle the progressive, physiological disorder involving the circulatory system primarily. (Roome; Bell, Clark and Cuthbertson; Krogh; Meek; Harkins; Moon.) Increase in capillary permeability in this connection refers to a continuous leakage of fluid and plasma proteins from the vascular system which perpetuates the oligemia and leads to the irreversible stage of shock followed by death. The chief findings which are attributed to an alteration of the normal capillary permeability in shock are *a*, progressive hemoconcentration; *b*, increased movement of fluid across the vascular membrane observed in isolated capillary beds, and *c*, the presence of edema revealed by histological examination of tissues at autopsy. Such evidence has been presented mainly in the form of a qualitative description.

The present study had for its objective the quantitative evaluation of normal capillary permeability, and the determination of the effect of traumatic shock upon it. Such evaluations call for precise definitions, and it soon became apparent that the expression "capillary permeability" is used very loosely. This ambiguity is easily understood when one considers the numerous factors which may influence the rate of passage of substances across the capillary membrane: 1, the size of particles or molecules which will pass through the capillary wall; 2, body temperature as it affects capillary endothelium; 3, hydrogen ion concentration of the tissue fluids which affects the electrical potentials of the capillary membrane; 4, the area of vascular surface available for interchange between blood and extravascular fluid; 5, the rate of blood flow through the capillaries; 6, the relative size of the vascular and extravascular fluid compartments available for the dilution of various substances; 7, the balance between colloid osmotic pressure and capillary blood pressure; 8, the magnitude of tissue pressure which controls excessive filtration of fluid; 9, the activity of hormones which may aid specifically in the control of the exchange of fluids, ions and molecules across the capillary wall. Admittedly this list must be incomplete. These factors, which are not necessarily independent but may be intimately correlated, are influential in determining the rate of transcapillary movement under normal conditions; in pathological states, the effect of noxious

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² Henry Strong Dennison Scholar, 1939-40; 1940-41.

agents on the capillary membrane, anoxemia, acidosis and alkalosis, etc., may also be significant in producing an alteration in this rate.

Krogh has defined the absolute capillary permeability as the limit of size of particles and molecules which will just pass through the capillary wall. In traumatic shock there is an increase of the absolute capillary permeability as evidenced by the excessive loss of plasma proteins. Whether this alteration is generalized or merely localized to the site of injury has not been definitely settled.

In any case, since absolute capillary permeability is only one of many factors influencing the physiological exchange of substances across the capillary membrane, it is profitable to consider the membrane not as something divorced from the rest of the body but as a part of the system in which it is operating. The rate of movement of substances which cross the capillary membrane freely in normal conditions gives a quantitative measure of normal exchange and a base line for evaluating a similar measure under pathological conditions such as shock. Any alteration in the rate which may be observed in shock may be due to a change in one factor or several in the system. The experiments to be described led to an estimation of the rate of exchange of ionic sodium between the plasma and extravascular fluids in the normal dog, in the animal in shock, and in the shocked dog following replacement therapy.

MATERIALS AND METHODS. *Normal.* The determination of the rate of exchange of sodium between the plasma and extravascular fluids was based upon two separate series of experiments, one pertaining to the time change of the concentration in the plasma of intravenously injected radioactive sodium, and the other to the time change in concentration in other parts of the body. The chief evidence was obtained from the observations on plasma concentration, and these will be described first.

Sixty-four experiments were performed on 43 mongrel, adult dogs. Preliminary experiments indicated that there was no perceptible effect on the results when animals were anesthetized with ether, morphine, or nembutal; in the main body of experiments, therefore, intravenous nembutal (30 mgm. per kilo. body weight) was used. The saphenous artery and femoral vein were exposed. Radioactive sodium present as the chloride (Na^{24}Cl) was injected into the femoral vein in strengths varying from 0.1 millicurie to 0.3 millicurie. Samples of blood varying from 2 cc. to 5 cc. were removed by arteriopuncture. Since blood samples were removed at short intervals, it was important to avoid stasis of the circulation at the site of removal. The saphenous artery, a small but constant branch of the femoral artery (Ellenberger and Baum), was therefore exposed at its origin just proximal to the entrance of the femoral artery into Hunter's canal. By inserting the needle first into the saphenous artery and then passing it into the femoral artery, it was possible to sample the main femoral arterial blood without at any time having to impede the circulation in order to control bleeding. The measurement of the radioactivity was made on 1 cc. samples of plasma in a pressure ionization chamber string electrometer apparatus (Flexner and Pohl).

The observations on the time change of concentration of radioactive sodium in the plasma were made at intervals from 0.5 minute after intravenous injection of Na^{24}Cl to 120 minutes. The mixing time of intravenously injected dyes has been reported as varying from 5 minutes to 40 minutes in the dog. Since a major portion of the equilibration reaction of intravenously injected Na^{24} takes place within the first 5 minutes after its injection, it was important to establish its mixing time in order to know when observations were valid for determining the rate of equilibration. Two issues were examined relative to this question: 1, the point in time at which observations first showed stability as evidenced by lying on the time concentration curve which described later values, and 2, the time interval after injection at which simultaneous samples of blood secured from widely separated areas of the body showed identical concentrations of Na^{24} . Thirty-eight experiments were performed in which samples of blood were secured by arteriopuncture 30 seconds after injection of the radioactive sodium and at subsequent times up to 120 minutes. In every instance, the value at one-half minute fell on the time-concentration curve which described the later values. In three experiments, simultaneous samples of blood were taken from the femoral artery of one extremity and the femoral vein of the other extremity from one half minute after injection of Na^{24}Cl to 120 minutes. The concentrations of radioactivity present in the arterial and venous plasma were, within the limits of error of measurement (3 per cent), indistinguishable in all samples. We concluded therefore that the mixing of Na^{24} was complete at one-half minute after intravenous injection in the normal dog.

In order to complement the observations on plasma concentration, observations were made on the change in concentration of Na^{24} with respect to time in the extravascular fluids. This was done by determining the distribution of Na^{24} in selected tissues at various time intervals after injection. Radioactive sodium was injected intravenously in 18 dogs under nembutal anesthesia; after a known time interval they were sacrificed by decapitation. They were suspended in the air by their feet in order to facilitate the rapid drainage of the blood, and the tissues to be examined were removed as expeditiously as possible in order to prevent postmortem shifts of electrolytes. After weighing the tissues, they were ashed at red heat with sulfuric acid. The radioactivity present in the ash was measured by the apparatus previously described.

Untreated shock. Shock was produced by muscle crushing according to the technique described by Duncan and Blalock. Approximately 800 pounds of pressure were applied to a ridged press which stretched and crushed the thigh muscles of one leg. The animals were anesthetized with intravenous nembutal (30 mgm./kgm. body wt.). Supplementary narcosis was provided by subcutaneous morphine and/or nembutal when indicated. The shock press was routinely applied for 5 hours. No sedation was given after the fourth hour. One hour after the removal of the press, Na^{24} was given intravenously and samples of blood secured as in the normal series. The time for complete mixing of Na^{24}Cl in shock, estimated in the same way as in the normal series, was found to be approximately one minute after intravenous injection.

In preliminary experiments it had been found that within the first hour after removing the press the blood pressure declined 30 to 50 mm. Hg. In our experience, 70 per cent of the animals shocked by this method expired within 8 hours after discontinuation of the trauma; 90 per cent were dead within 24 hours. The remaining animals died in several days from gas gangrene infections. This agrees well with the observations of Duncan and Blalock. Because of the uniformity with which shock was produced by this method, as indicated by the survival time, it was not thought necessary to carry out extensive laboratory procedures to confirm the diagnosis of shock. Such easily observed clinical features as the character of the respirations, the pulse rate, the appearance of the mucous membranes and the external body temperature, provided a satisfactory measure of the depth of shock in the untreated animals. Twenty-nine dogs were used for the determination of the rate of sodium exchange.

The distribution of Na^{24} in the tissues at various time intervals after intravenous injection was determined as in the normal dogs. Shock was produced by muscle crushing, and the experiments were routinely started one hour after removal of the press. Fifteen dogs were used in these determinations.

Treated shock. In the determination of the effect of therapy on the rate of sodium exchange between plasma and extravascular fluids in shock, saline and serum were chosen as the therapeutic agents. Ten animals were used in each series. Shock was produced in the usual manner. One hour after removal of the press, a volume of isotonic saline or fresh dog serum equal to 5 per cent of the animal's body weight was given intravenously. Serum was administered rather than plasma in order to avoid any possible effects by anticoagulants on the rate of exchange. The blood pressure and a hematocrit reading were taken immediately before the parenteral fluid administration, and repeated 10 minutes after the completion of the therapy. Radioactive sodium was injected intravenously immediately thereafter and samples of blood secured in the usual manner.

The clinical appearance of the animals showed a striking improvement following the replacement therapy. In each instance the animal roused from his semi-stuporous state. The mucous membranes of the mouth, which invariably were cold and pale in the untreated animal, became pink, moist, and warm. The hematocrit reading showed the expected drop to normal or below normal levels. The blood pressure response in the saline treated animals was less spectacular. In two of the animals receiving saline, the pressure remained unchanged. In the other dogs the mean systolic pressure was increased from 10 mm. Hg to 50 mm. Hg over the level just prior to venoclysis, and the average increase for the entire group was just 15 per cent. The mean systolic blood pressure of the animals receiving serum showed a more satisfactory response to the treatment. The pressure rose in every case and the average increase was 35 per cent over the pretreatment readings. In spite of the improved clinical appearance, 19 out of the 20 animals died within 48 hours.

RESULTS. *Normal.* The time change in concentration of radioactive sodium in the plasma in the normal animal following intravenous injection has been

studied in 64 experiments and has been found in every case to have the same general form, namely, a J-shaped curve with a steady decline to equilibrium. From 90 minutes after injection to 120 minutes the concentrations remain level, and the 120 minute value was therefore considered the equilibrium concentration. It is recognized that this is not a true equilibrium, for penetration of certain tissues, such as bone and the erythrocytes, continues to take place for 24 hours at least. Relative to the reaction we are considering, the continued slow penetration of Na^{24} into such tissues over a period of 24 hours plays a negligible rôle and therefore the rate at which the concentrations approach the level attained at 120 minutes has been analyzed.

In order to get greater stability from the observations than was possible in a single experiment, the observations from all 64 experiments were averaged. Before doing this, the experiments were examined for certain issues with regard to homogeneity of the material, for under some conditions of variability it is possible to have an average curve which is not characteristic of the individual experiments. An analysis of 32 rate determinations made on 11 dogs, where repeat rates were performed from 2 hours to 10 days after the initial rate determination, showed that the repeat rates determined on the same animal were as variable as rates determined on different animals. This, together with the fact that the average of the individual rates was essentially the same as the rate determined by averaging the observations, indicates that it was possible to average without doing violence to the material.

The difference in the weights of the animals and in the amount of Na^{24} injected necessitated putting all of the observations on a common basis: This was done by scaling the observed concentration at 120 minutes to 1000 β -rays per sec. per cubic centimeter plasma for every animal and adjusting all of the other concentrations accordingly. Since 120 minutes was also taken as the "equilibrium" time, 1000 became the equilibrium value.

Plotted in figure 1, it is seen that the observed points form a very smooth pattern. The fitted curve, from which the calculated values of table 1 are derived, is discussed below. It was the objective of the quantitative analysis to study the rate of equilibration of these concentrations and to see what this implied as to the rate of transfer of sodium across the capillary membrane.

The analysis reveals at once that more than a single rate is involved because the difference between the observed concentrations and the equilibrium value is not linear on logarithmic paper. It was found, however, that two rates sufficed to describe the entire series of concentrations from 0.5 minute to 120 minutes.

In considering the way sodium may be exchanged across the capillary and cell membranes to account for these two rates, three hypotheses were examined: first, that there is another reservoir besides the plasma and interstitial fluid into which Na^{24} is distributed at a different rate from the transcapillary exchange; second, that the sodium passes across the vascular membrane into the interstitial fluid and then across the cell membrane, these two transfers taking place at different rates; third, that the two rates are produced by a differential rate of

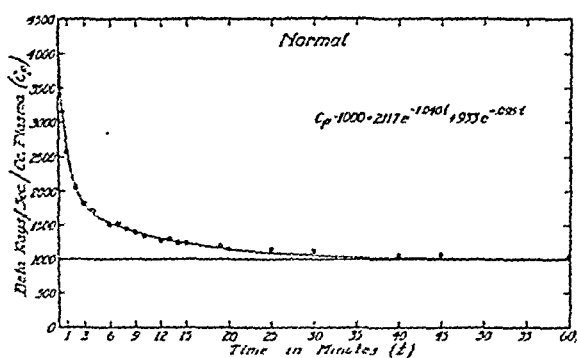


Fig. 1

Fig. 1. Time change in plasma concentration of Na^{24} following intravenous injection in normal dogs.

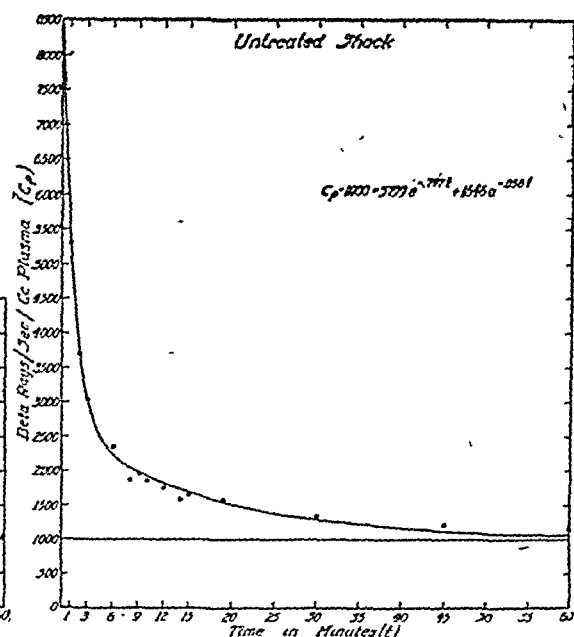


Fig. 2

Fig. 2. Time change in plasma concentration of Na^{24} following intravenous injection in untreated shocked dogs.

TABLE 1
Time change in concentration of Na^{24} in plasma—normal

TIME AFTER INJECTION	NUMBER OF OBSERVATIONS	AVERAGE CONCENTRATION OF Na^{24} IN PLASMA (β -RAYS PER SEC. PER CC.)	
		Observed	Calculated
min.			
0			4050
0.5	38	3162	3148
1	59	2575	2597
2	59	2050	2036
3	48	1802	1795
4	18	1713	1671
6	45	1508	1532
7	11	1528	1481
8	22	1433	1437
9	28	1392	1397
10	29	1341	1361
12	47	1271	1298
13	11	1292	1271
14	23	1233	1247
15	28	1236	1224
19	10	1199	1153
20	10	1140	1140
25	16	1145	1087
30	9	1113	1051
40	6	1053	1021
45	30	1063	1013
60	39	1030	1003
120	64	1000	1000

transfer across the capillary wall in different parts of the system. Various experiments were carried out to produce evidence on this matter.

Our first tentative hypothesis was that the erythrocytes constituted an additional reservoir and that the exchange of Na^{24} across the red cell membrane was at an entirely different rate from that of the exchange between plasma and interstitial fluid. To test this, the amount of penetration of Na^{24} into the erythrocytes was determined at intervals after intravenous injection, and also by *in vitro* experiments; it was found to be appreciable during the time interval of the experiments (2 hrs.). To assess the importance of the red cell penetration in accounting for the observed concentration curves, the rate of disappearance of Na^{24} from the blood stream of dogs with drastically reduced red cell volumes was studied. Three animals were bled, the blood loss being replaced by an equal volume of plasma until the hematocrit was reduced 50 per cent or more. In one dog, the hematocrit was reduced to 6 to 8 per cent by controlled hemorrhage and infusion of hemoglobin-Ringer solution prepared according to the technique of Amberson. In every case, the rate of disappearance of Na^{24} proceeded along a double exponential curve which was essentially the same as the control curve. From these experiments we concluded that the penetration of Na^{24} into the red blood cells is not an appreciable factor in accounting for the two rates.

The second hypothesis tested was that other cells besides the erythrocytes constituted an additional reservoir for the Na^{24} and that the two observed rates were explained by an exchange of Na^{24} between the plasma and the interstitial fluid, followed by an exchange at a different rate between the interstitial fluid and the cells. This hypothesis was difficult to examine directly, but various indirect tests were applied. We assumed that chloride is "essentially" an extracellular ion. "Essentially" is used advisedly because Manery and Hastings, Amberson and others have presented evidence which indicates that in certain tissues chloride is both intra- and extracellular. The argument was, then, that if the two rates observed in the disappearance of Na^{24} from the blood stream were due to its passage first into the extracellular fluid and from there into the water of the body cells, the disappearance of the "essentially" extracellular chloride ion from the blood stream should proceed at a single exponential rate. In one experiment, radioactive chloride present as the sodium salt (NaCl^{38}) was injected intravenously and the curve of equilibration in the plasma was found to involve two rates. Because of the difficulty of working with radioactive chloride ions (half-life 37 min.), radioactive bromine was substituted, since it has the same distribution in the animal body as the chloride ion (Wallace and Brodie). The rate of equilibration of radioactive bromine (Br^{82}) was studied in three experiments. In each case the observations were described by a double exponential curve. These experiments did not deny the possibility of the hypothesis to be tested, but they suggested that some other explanation was involved.

The third hypothesis, that there is a differential rate of transfer across the vascular membrane in different parts of the body, was examined in two ways.

In four experiments, the disappearance of intravenously injected Na^{24} from the blood stream and, simultaneously, its appearance in the cerebrospinal fluid, were observed. In these experiments, the curve describing the appearance of Na^{24} in the spinal fluid showed a very gradual rise toward equilibrium when compared with the steep decline in the blood concentrations. These experiments confirm the similar observations made by Greenberg, Aird, Boelter, Campbell, Cohn and Murayama. Even more illuminating was the study of the distribution of Na^{24} in the body at intervals after injection. This will be presented in detail in a later part of the paper. These experiments indicated that the third hypothesis was acceptable and it is therefore the basis for the interpretation of the form of the relationship of change of concentration of radioactive sodium in the plasma and time.

A restatement of the hypothesis is that, whatever the variation in rates of exchange of sodium across the capillary membrane in different parts of the body, two distinct rates are both necessary and sufficient to summarize the principal features of the exchange throughout the system. It is obvious that in any system as involved as the capillary, such a hypothesis is an oversimplification, but it serves to describe the major effects.

It may be noted that the theory which results from this hypothesis is a direct extension of that developed for the transcapillary exchange of water and of sodium in the guinea pig where only a single rate of transfer of the substance was postulated (Flexner, Gellhorn and Merrell).

The derivation of the relationship between sodium exchange and change in concentration of radioactive sodium in the plasma under the present hypothesis will now be developed as a basis for the subsequent presentation of the results.

It is assumed that sodium ions are exchanged between the plasma and extravascular fluids by means of a constant number passing across the capillary wall in each direction per unit of time. This assumption does not mean that the rate of transfer is the same for all portions of the capillary bed, but merely that the overall amount of sodium exchanged per unit of time is constant.

It is assumed further that the variability of rates throughout the system is such that two average rates suffice to summarize the exchange.

Then the sodium ions in the extravascular system may be considered as subdivided into two groups as far as rate of exchange is concerned, one group being in various areas, A , where the rate of exchange with the plasma ions is the more rapid of the two, and the other in various areas, B , where the rate of exchange with the plasma ions is appreciably slower.

The following notation will be used in the mathematical discussion and presentation of results.

1. Distribution of sodium in the body.

n_p , n_A and n_B = number of sodium ions in the plasma, area A and area B respectively

$n_T = n_p + n_A + n_B$ = total number of sodium ions in the body

$k_p = \frac{n_p}{n_T}$, $k_A = \frac{n_A}{n_T}$, $k_B = \frac{n_B}{n_T}$ represent the proportions of the total sodium

which are in the plasma, area A and area B respectively.

2. Amount exchanged per unit of time.

r_A = number of sodium ions passing from plasma to area A or from area A to the plasma per unit of time

r_B = number of sodium ions passing from plasma to area B or from area B to the plasma per unit of time

$r_A + r_B$ = number of sodium ions leaving plasma or number entering plasma per unit of time

3. Proportion of sodium exchanged per unit of time.

$R_{pA} = \frac{r_A}{n_p}$, $R_{pB} = \frac{r_B}{n_p}$, and $R_p = \frac{r_A + r_B}{n_p}$ represent the proportions of plasma sodium passing respectively into area A , area B , and the total extravascular area per unit of time.

$R_A = \frac{r_A}{n_A}$, $R_B = \frac{r_B}{n_B}$ = proportions of sodium in area A and in area B respectively passing into plasma per unit of time.

$R_{TA} = \frac{r_A}{n_T}$, $R_{TB} = \frac{r_B}{n_T}$, and $R_T = \frac{r_A + r_B}{n_T}$ represent the proportions of total sodium exchanged in either direction between the vascular system and respectively area A , area B and the combined extravascular area, per unit of time.

Now let a group of radioactive sodium ions, N_0 , be injected into the plasma at a point in time, $t = 0$, the number, N_0 , being sufficiently small relative to the amount of ordinary sodium to leave the total number of ions essentially unchanged. These ions will behave in exactly the same way in rates of exchange as the other sodium ions, but their identification will allow these rates to be determined through observing the rate at which they attain the normal distribution throughout the system.

Let N_p , N_A and N_B = the respective numbers of radioactive ions in each area at time t , measured from $t = 0$.

$$\text{Then } N_p + N_A + N_B = N_0 \quad (1)$$

Under the assumptions stated above, the change in N per unit of time in each area is given by

$$\left. \begin{aligned} \frac{dN_p}{dt} &= -r_A \frac{N_p}{n_p} + r_A \frac{N_A}{n_A} - r_B \frac{N_p}{n_p} + r_B \frac{N_B}{n_B} & (a) \\ \frac{dN_A}{dt} &= r_A \frac{N_p}{n_p} - r_A \frac{N_A}{n_A} & (b) \\ \frac{dN_B}{dt} &= r_B \frac{N_p}{n_p} - r_B \frac{N_B}{n_B} & (c) \end{aligned} \right\} \quad (2)$$

Dividing each of these equations by the appropriate volume available for dilution of sodium to get change in concentration of radioactive sodium, and integrating,³ we obtain the following relationship of plasma concentration, c_p , and time.

$$c_p - c_{eq} = a_1 e^{b_1 t} + a_2 e^{b_2 t} \quad (3)$$

³ For the steps in the solution, see the Appendix.

where c_{eq} = the plasma concentration of Na^{24} at equilibrium, b_1 and b_2 are rates, and a_1 and a_2 concentrations dependent solely on the movement and distribution of ordinary sodium.

The constants in equation (3) pertain to the concentration of Na^{24} in the plasma, but they can be converted into the constants defined above for the distribution and exchange rates of ordinary sodium. The following equations permit the passing from one set of constants to the other.

$$b_1 + b_2 = -(R_p + R_A + R_B) \quad (4)$$

$$\frac{c_{eq}}{c_0} (b_1 b_2) = R_A R_B \quad (5)$$

$$\frac{-a_1 b_1 - a_2 b_2}{c_0} = R_p \quad (6)$$

$$\frac{c_{eq}}{c_0} = k_p \quad (7)$$

where c_0 is the value of c_p at $t = 0$.

From the fundamental definitions of the rate and distribution constants previously given, other constants for ordinary sodium can be obtained in terms of those given by equations (4) to (7), namely

$$R_{pA} = \frac{R_p - R_B \left(\frac{1}{k_p} - 1 \right)}{1 - \frac{R_B}{R_A}} \quad (8)$$

$$R_{pB} = R_p - R_{pA} \quad (9)$$

$$R_T = R_p \cdot k_p \quad (10)$$

$$k_A = \frac{R_{pA}}{R_A} \cdot k_p \quad (11)$$

$$k_B = \frac{R_{pB}}{R_B} \cdot k_p \quad (12)$$

Equation (3) indicates that under the conditions of exchange postulated, the concentration of radioactive sodium in the plasma approaches equilibrium at a double exponential rate.⁴ Equations (4) to (12) show that the rates of approaching equilibrium for the radioactive sodium are dependent solely on the proportion of ordinary sodium transferred per minute, and the relative amounts of sodium present in the various areas.

It is this fact that creates the real value of equation (3). The constants of the equation may be determined by curve fitting processes to observations on radioactive sodium concentrations, but their importance to us is that they may be used as intermediate computing constants to derive the rates of exchange and distribution of sodium ions.

⁴ Equations relating c_A and c_B to time are also double exponentials with the same values of b_1 and b_2 as in equation (3), but different values of a_1 and a_2 .

Turning now to the application of this theory to our specific problem, it is necessary first to fit equation (3) to the observations of table 1 and then to calculate, from the arithmetic values of the constants, the rates of sodium exchange and the proportion of the total sodium distributed in each of the three areas. In order to get as objective a fit as possible to the observations, the method of least squares was used, minimizing vertical deviations, weighted according to the number of observations involved in each measurement. The resulting equation is:

$$c_p - 1000 = 2117e^{-1.040t} + 933e^{-0.095t}$$

This is plotted against the points in figure 1 and is seen to give a very satisfactory description of them.

Substituting the values of the constants thus obtained from the fitted curve into equations (4) to (12) leads to the values for the rates of exchange of sodium in the three areas, and the proportion of the total sodium present in each area. These are given in table 7. The explanation and discussion of them will be postponed until the results of the untreated shock and treated shock experiments have been presented, because the salient features are best brought out by a comparison of all the results.

Before turning to the results of the shock experiments, we shall consider the distribution of radioactive sodium in the tissues of the normal animal at various time intervals after injection. As stated in the section on Methods, the purpose of these experiments was to gain an estimate of the rate of change in concentration of Na^{24} in the extravascular fluids with respect to time. The results given in table 2 represent the averages of repeated experiments at each time.

TABLE 2

Na^{24} distribution in normal dogs at various times after injection¹

AVERAGE* RATIO OF Na^{24} PER GRAM TISSUE TO Na^{24} PER CC. OF PLASMA

TISSUE	TIME AFTER INJECTION					
	$\frac{1}{2}$ min.	5 min.	30 min.	1 hr.	2 hrs.	24 hrs.
Muscle.....	0.120	0.130	0.142	0.187	0.131	0.149 ^(b)
Lung.....	0.262	0.430	0.422	0.422	0.354	0.478 ^(b)
Intestine.....	0.177	0.259	0.312	0.266	0.269	0.344 ^(b)
Liver.....	0.106	0.155	0.167	0.145	0.178	0.196 ^(b)
Skin.....	0.082	0.320	0.430	0.410	0.388	0.405 ^(b)
Tendon.....	0.039	0.332	0.470	0.431	0.377 ^(a)	
Bone.....	0.087	0.101	0.266	0.382	0.388 ^(a)	0.597 ^(b)
Brain.....	0.009	0.050	0.028	0.063	0.122	0.197

* Average of 3 experiments except for: (a) average of 2 experiments; (b) average of 4 experiments.

Each value is the average ratio of the amount of Na^{24} in a gram of tissue to the amount in a cubic centimeter of plasma at a given time. Thus an increasing ratio with time means that the concentration in the extravascular fluid is in-

creasing relative to that of the plasma, whereas a constant ratio means that the change in extravascular concentration is just that in the plasma, that is, the two are in equilibrium.

From an examination of the average ratio of a particular tissue at successive time intervals, the following information emerges. The various tissues analyzed may be roughly divided into two groups. In one group comprised of muscle (the thigh muscles were the routine site of sampling), lung, intestine and liver, the radioactive sodium is very rapidly distributed throughout the extravascular fluids as indicated by a virtual stabilization of the ratio of tissue concentration of Na^{24} to plasma concentration from 5 minutes to 2 hours. In the other group, consisting of skin, tendon, bone, and brain, the rate of equilibration of Na^{24} between the plasma and extravascular fluids is appreciably slower. In this group, the ratio of the concentration of Na^{24} in the tissues to that in the plasma tends to increase throughout the 2-hour interval. In every tissue examined there is an increase in the ratio from 2 hours to 24 hours, although it is more marked in the brain and bone than in the other tissues. These observations made 24 hours after injection demonstrate that 120 minutes is not a true equilibrium point, but they also show that within 2 hours the major portion of the reaction toward equilibrium has taken place.

The observations of table 2 give support to the hypothesis that radioactive sodium leaves the blood stream at more than one rate. It is possible that in the interpretation of the rates of exchange, area *A* is composed of such tissues as muscle, lung, intestine, and liver where the exchange of sodium between the plasma and extravascular fluid is rapid, and area *B* consists of skin, tendon, spinal fluid and bone, where the transfer is appreciably slower.

Untreated shock. It is unnecessary to develop a new hypothesis for the exchange of sodium in the shocked animal because the observations on the time change of concentration of radioactive sodium in shock show a pattern similar to those of the normal animal, namely, a decline in concentration toward equilibrium along a double exponential curve. Nevertheless, it is a well established fact that hemoconcentration is characteristic of traumatic shock. This indicates that there is not a constant rate of exchange across the capillary membrane, but that more of the fluid portion of the blood leaves the vascular system than is returned from the extravascular system to the blood per unit of time. However, due to the magnitude of the transcapillary exchange, this differential in the overall rate must be so small in 2 hours that it does not affect our results or vitiate the hypothesis upon which we are operating. As in the normal animals, in order to put the 29 shock experiments on a common basis, the concentration at 120 minutes was made equal to 1000 β -rays per second per cubic centimeter, and all of the other concentrations adjusted accordingly. The average concentrations, together with the number of observations made at each time interval, are given in table 3. As before, the equation was fitted to the observed points by considering the 2-hour value as the equilibrium value and fitting the remaining constants by least squares. (See figure 2.)

The resulting equation is:

$$c_p - 1000 = 5799e^{-0.747t} + 1646e^{-0.058t}$$

The arithmetic values of a_1 , a_2 , b_1 and b_2 were substituted in equations (4) to (12) to obtain the rates of exchange of sodium across the capillary wall and also the proportions of the total sodium present in the various areas. These appear in table 7 and will be discussed presently. It will be sufficient here to note that the results show a decreased rate of transcapillary exchange of sodium in the shocked animal.

TABLE 3
Time change in concentration of Na^{24} in plasma—untreated shock

TIME AFTER INJECTION <i>min.</i>	NUMBER OF OBSERVATIONS	AVERAGE CONCENTRATION OF Na^{24} IN PLASMA (β -RAYS PER SEC. PER CC.)	
		Observed	Calculated
0			8415
1	21	5322	5301
2	25	3706	3767
3	22	3043	3000
6	22	2353	2228
8	6	1871	2050
9	21	1965	1984
10	5	1854	1925
12	23	1765	1821
14	6	1587	1731
15	20	1663	1690
19	10	1575	1547
30	16	1326	1289
45	11	1205	1121
60	20	1136	1051
90	20	1058	1009
120	29	1000	1002

The distribution of Na^{24} in the tissues at intervals after injection provides confirmatory evidence to the rate studies and also emphasizes the difference from the normal. The average ratio of the concentration of Na^{24} in the tissues to that in the plasma is presented in table 4. The most striking points brought out by these experiments are first, the order of the tissues as to speed of equilibration which is in general the same as in the normal animal and second, the prolongation of the time interval before the ratios show stability. The ratios in the shocked animal at 5 minutes are in general the same as in the normal animal at $\frac{1}{2}$ minute. There also is more tendency in the shock experiments for a continued rise in the ratio throughout the 2-hour period. In this connection, it is worth noting that the ratios for the various tissues at 2 hours are consistently greater in the shocked animal than in the normal. This might be ex-

plained by various ones of the theories concerning shock. Thus a generalized leakage of the plasma into the tissues perceptible at 2 hours would explain it, as would also the existence of trapped blood so that the tissue of the shocked animal was less free of blood when the measurements were taken. No collateral evidence is at present available to throw light on this point.

It is of interest to compare the actual ratios of the normal muscle and the muscle which has been traumatized. The consistently increased ratios in crushed muscle are due to the loss of plasma into the traumatized area and also probably to penetration of the injured muscle cells by radioactive sodium.

TABLE 4

Na²⁴ distribution in shocked dogs at various times after injection
AVERAGE* RATIO OF Na²⁴ PER GRAM TISSUE TO Na²⁴ PER CC. OF PLASMA

TISSUE	Time after injection			
	5 min.	30 min.	1 hr.	2 hrs.
Normal muscle....	0.055	0.111	0.152	0.173
Crushed muscle ..	0.113	0.370	0.400	0.712
Lung	0.331	0.352	0.458	0.496
Intestine . . .	0.207	0.256	0.242	0.330
Liver.	0.145	0.151	0.182	0.240
Skin	0.104	0.318	0.317	0.517
Tendon	0.033 ^(a)		0.174 ^(a)	
Bone..	0.095	0.351	0.342	0.478
Brain .	0.009	0.035	0.072	0.138

* Average of 3 experiments except for: (a) average of 2 experiments.

Treated shock. The same hypothesis that was applied to the normal and untreated shock experiments also forms the basis for the interpretation of the experiments on shocked dogs following replacement therapy. Table 5 presents the average concentration of Na²⁴ in the plasma in 10 shocked dogs following parenteral saline administration. The fitted equation to these observations, plotted against the observed concentrations is shown in figure 3, and has the form:

$$c_p - 1000 = 2597e^{-0.427t} + 863e^{-0.033t}$$

The application of the theory to the arithmetic values of the constants thus obtained is presented in table 7, and will be discussed later.

Table 6 and figure 4 present the average concentrations of Na²⁴ in the plasma of 10 shocked dogs following serum therapy, and the curve fitted to these observations. The equation for the concentration of intravenously injected radioactive sodium is

$$c_p - 1000 = 2590e^{-0.546t} + 773e^{-0.047t}$$

As in the three previous series of experiments, the constants of this curve have been interpreted in terms of the rate of exchange of ordinary sodium and of the proportion of the total sodium in the three areas, presented in table 7.

Comparison of the results in normal and shocked animals. The constants in table 7, with the exception of c_0 , refer to ordinary sodium, not to radioactive sodium. The double exponential observed for the tracer substance is the consequence of the transcapillary movement of ordinary sodium. Under the state-

TABLE 5
Time change in concentration of Na^{24} in plasma—shock + saline

TIME AFTER INJECTION min.	NUMBER OF OBSERVATIONS	AVERAGE CONCENTRATION OF Na^{24} IN PLASMA (β -RAYS PER SEC. PER CC.)	
		Observed	Calculated
0			4460
1	8	3543	3525
2	10	2863	2905
3	10	2514	2491
6	9	1879	1887
9	10	1698	1669
12	10	1563	1562
15	10	1495	1492
19	9	1365	1420
25	5	1329	1334
30	5	1201	1276
35	5	1286	1228
45	10	1158	1156
60	10	1099	1088
90	10	1073	1028
120	10	1000	1009

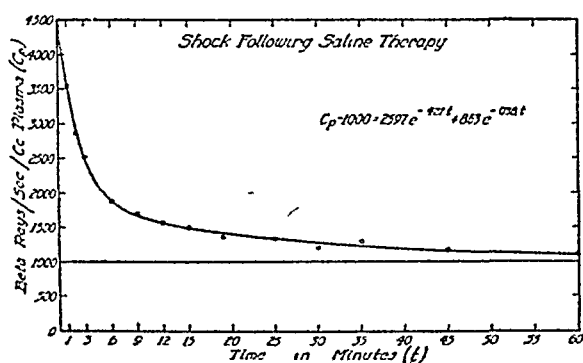


Fig. 3

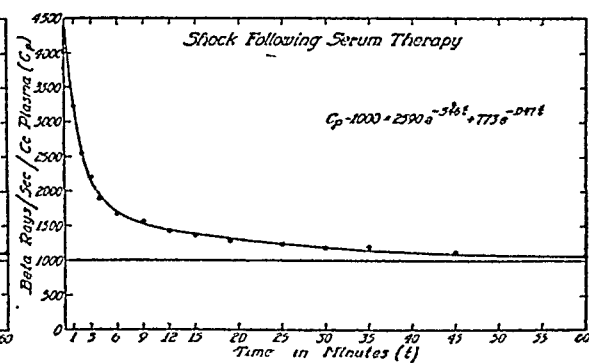


Fig. 4

Fig. 3. Time change in plasma concentration of Na^{24} following intravenous injection in shocked dogs given saline treatment.

Fig. 4. Time change in plasma concentration of Na^{24} following intravenous injection in shocked dogs given serum treatment.

ment of this movement, given earlier in the paper, the constants of the fitted curves can be turned into constants describing the distribution and rate of movement of ordinary sodium.

The basis for getting at the distribution of Na is the ratio of initial concen-

TABLE 6
Time change in concentration of Na^{24} in plasma—shock + serum

TIME AFTER INJECTION <i>min.</i>	NUMBER OF OBSERVATIONS	AVERAGE CONCENTRATION OF Na^{24} IN PLASMA (β -RAYS PER SEC. PER CC.)	
		Observed	Calculated
0			4363
1	9	3246	3238
2	10	2545	2573
3	10	2205	2175
4	4	1899	1932
6	9	1675	1681
9	10	1562	1525
12	9	1424	1443
15	10	1369	1383
19	10	1285	1317
25	4	1231	1239
30	6	1181	1189
35	4	1185	1149
45	4	1119	1093
60	10	1063	1046
90	10	1020	1011
120	10	1000	1003

TABLE 7

Constants giving sodium distribution and rate of exchange in normal dogs, untreated shock, and treated shock

CONSTANT	NORMAL	UNTREATED SHOCK	SHOCK + SALINE	SHOCK + SERUM
Initial plasma conc. of Na^{24} , where c_{eq} is 1000 β -rays/sec/cc. c_0	4050	8445	4460	4363
Na in plasma/total Na k_F	0.247	0.119	0.224	0.229
Na in area A/total Na k_A	0.219	0.185	0.249	0.271
Na in area B/total Na k_B	0.534	0.696	0.527	0.500
% of Na in plasma transferred per min. from plasma to extravasc. fluid R_F	56.6	52.4	25.6	33.2
plasma to area A R_{PA}	46.5	40.9	21.1	27.8
plasma to area B R_{PB}	10.1	11.5	4.5	5.4
% of Na in total body transferred per min. from plasma to extravasc. fluid R_T	14.0	6.2	5.7	7.6
plasma to area A R_{TA}	11.5	4.8	1.7	6.1
plasma to area B R_{TB}	2.5	1.4	1.0	1.2

tration of Na^{24} in the plasma to the equilibrium concentration. The initial concentration cannot be obtained by direct observation because of lack of mixing but can be obtained by extrapolation of the fitted curve back to $t = 0$. A com-

parison of c_0 for normal and shocked animals shows that the normal value is only half as large as that for untreated shock, but is only slightly smaller than the values for treated shock. This is due to the fact that in untreated shock the plasma volume is reduced, and indicates that the reduction is about 50 per cent. In the treated animals the plasma volume is brought back to nearly normal levels.

In this connection it is of interest to note that in a series of 44 individual normal experiments, the extrapolated c_0 values, together with the known amount of Na^{24} injected and the body weight, were used to estimate plasma volume. On the average, the amount injected (on the basis of an equilibrium value of 1000 β -rays per sec. per cc. plasma) was 36,170 β -rays per sec. per 100 grams body weight. The average c_0 was 4224 β -rays per sec. per cc., yielding a plasma volume of 8.6 cc. per 100 grams. Since this volume is about twice that estimated by dye methods (Smith, Arnold and Whipple, Gregersen), it would seem that either the reaction in the first half minute is not described by our equation, or that we are not accounting for all the Na^{24} injected. In either case, the c_0 values are valid in a *relative* sense in describing changes in plasma volume in shock. Since the plasma volumes are too high in absolute value, the proportion of sodium allocated to the plasma is also too high but, again, the relative comparison between normal and shock holds.

The proportion of the body sodium which is in the plasma is given by k_p , that is, plasma sodium/total sodium. The values of k_p show that in the normal animal this is 25 per cent, whereas in untreated shock only 12 per cent is in the plasma. This is again associated with change in plasma volume, since in shock the total sodium in the body and the sodium concentration in the plasma are essentially unchanged. In treated shock, the proportion of the total sodium located in the plasma is back to normal, largely due to the restoration of the plasma volume.

The distributions of the extravascular sodium in the area of rapid exchange, A , and in the area of slow exchange, B , are indicated by k_A and k_B respectively. For the normal and treated shock, about 25 per cent of the sodium is in A and about 50 per cent in B . For the untreated shock, there is an increase in the proportion located in the area of slow penetration.

Turning now to the rates of exchange of sodium across the capillary membrane, we have first, R_p , which gives the proportion of plasma sodium exchanged per minute. For the normal animal, 56 per cent of the plasma sodium is exchanged per minute, for untreated shock 52 per cent, and for treated shock approximately 30 per cent. At first glance it would appear that there is no great difference in rate of exchange of sodium across the capillary wall in normal and traumatic shock, but that there is a marked reduction in rate when replacement therapy is used. However, this interpretation would be valid only if the conditions were the same as to sodium distribution. In a system where the proportion of sodium in the plasma is altered, an altered value of R_p would result if the rate of exchange remained the same. The essentially unaltered R_p in untreated shock indicates that there is a change in rate from the normal.

The real situation as to rate of exchange is perhaps best seen from the constant R_T , which gives the proportion of the total sodium in the body which is transferred across the capillary wall in each direction per minute. This is given by the product of R_p and k_p , thus it takes into account the change in distribution of sodium in the shocked animal. For the normal animal this is 14 per cent, and for the untreated shocked animal it is only 6.2 per cent. This indicates that a very profound change has occurred in the rate of exchange of sodium in shock, since there is a reduction of approximately 50 per cent in the total amount of sodium which is exchanged per minute.

Turning to the effect of replacement therapy on this rate, we see that the values are not brought back to normal levels, the saline treatment being associated with a 5.7 per cent rate and the serum treatment 7.6 per cent. In this case, since k_p is practically normal, the lowered rate is observed also in the values of R_p previously noted, about 50 per cent of the plasma sodium being exchanged per minute in the normal animal and about 25 to 33 per cent in treated shock. From these results it can be concluded that in traumatic shock there is a significant decrease in the amount of sodium that passes between the plasma and extravascular fluids per unit of time. Further, following fluid substitution therapy, the rate of exchange of sodium across the vascular membrane is not returned to normal, but remains at shock levels.

The remaining constants presented in table 7 bring out the fact that the reduction in the rate of exchange occurs in both the fast and slow rates. The values of R_{pA} and R_{pB} , showing the proportion of plasma sodium exchanged between area *A* and area *B* respectively are subdivisions of R_p and show the same relative difference between normal and shock as was seen in R_p . Similarly, they conceal significant alterations in the rates of exchange, which are made apparent when the alteration of sodium distribution is taken into account. If as before we multiply by k_p , we get the proportion of the total body sodium exchanged per minute between the plasma and area *A*, R_{TA} , and between the plasma and area *B*, R_{TB} . In both cases we see that the normal rate is about twice that of untreated shock, and the rates for shock following treatment remain at untreated shock levels.

SUMMARY

The object of the investigation was to determine whether traumatic shock produced an alteration in the normal physiological exchange of metabolites across the vascular membrane. To this end, the transcapillary rate of exchange of a substance to which the capillary membrane is freely permeable was studied.

The rate of equilibration in the plasma of a tagged ion, radioactive sodium, has been determined in the normal dog, in the animal in traumatic shock produced by muscle crushing, and in the shocked dog following intravenous saline and serum therapy. In each instance it was found that a double exponential curve consisting of a fast and a slow component was both necessary and sufficient to describe the observations. A hypothesis was developed to interpret the results of the experimental studies with radioactive sodium in terms of rates of

exchange of ordinary sodium across the capillary walls. Briefly, this hypothesis states that there are two rates at which sodium passes back and forth across the vascular membrane between the plasma and extravascular fluids in various areas of the animal body, the overall amount of sodium exchanged per unit of time remaining constant. Experiments on the distribution of radioactive sodium in the tissues at intervals after intravenous injection, and on the rate of appearance of intravenously injected radioactive sodium in the cerebrospinal fluid indicate that this hypothesis is acceptable, at least as a first approximation.

The experimental rate studies with radioactive sodium led to an estimate of the rates of exchange of ordinary sodium expressed as the proportion of the plasma sodium and the proportion of total body sodium which pass back and forth between the plasma and extravascular fluids per minute. These and certain other expressions of the rate of exchange of ordinary sodium have been presented and discussed in full in the foregoing sections. The conclusion to be drawn from them is that in untreated traumatic shock the total number of milligrams of sodium exchanged across the vascular membrane per unit of time is about 50 per cent of the normal. Immediately following replacement therapy with saline or serum in traumatic shock, the total amount of sodium that passes back and forth between the plasma and extravascular fluids is still only about 50 per cent of the normal value.

Numerous factors influence the rate of movement of ions and molecules across the vascular membrane in the normal animal. In a pathological condition such as traumatic shock there are probably additional circumstances which affect the rate. The most obvious explanation for the decreased rate of exchange of sodium in untreated shock is the inefficiency of the circulatory system as evidenced by a fall in blood pressure, prolonged circulation time, decreased cardiac output, hemoconcentration, etc. Following replacement therapy, these deficiencies are corrected temporarily at least. However, in spite of the obvious improvement in the animals' clinical appearance and in objective evaluations of the circulation (blood pressure, plasma volume, etc.), the experiments reported here indicate that the rate of exchange of sodium remains at shock levels. It is of interest to note in this connection that the 48-hour mortality rate of the treated animals (19/20) is not significantly different from that of the untreated animals.

It would be unjustified to conclude on the basis of these experiments that the reduced rate is attributable solely to changes in the capillary membrane since other parts of the capillary system may be the dominant influence. It is very likely that the condition of the systemic circulation is not reflected in the capillary bed and, therefore, the decreased rate of exchange of sodium is an expression of the reduced functional efficiency of the capillary circulation.

If the decrease in rate of movement of sodium across the vascular membrane is characteristic of other ions and molecules as well, it may be a fact of some consequence in understanding the changes in tissue metabolism observed in traumatic shock.

We are indebted to Dr. R. D. Evans, Dr. M. S. Livingston, and Dr. J. W. Irvine, Jr., for their generous co-operation in preparing the radioactive sodium in the Massachusetts Institute of Technology cyclotron.

APPENDIX. Solution of equations 2(a) and (b) of the text.

$$\frac{dN_p}{dt} = (-r_A - r_B) \frac{N_p}{n_p} + r_A \frac{N_A}{n_A} + r_B \frac{N_B}{n_B} \quad (2a)$$

$$\frac{dN_A}{dt} = r_A \frac{N_p}{n_p} - r_A \frac{N_A}{n_A} \quad (2b)$$

From the definitions of the rate constants (see text) and the relationship $N_0 = N_p + N_A + N_B$,

$$\frac{dN_p}{dt} = -R_p N_p + R_A N_A + R_B (N_0 - N_p - N_A) \quad (I)$$

$$\frac{dN_A}{dt} = R_{pA} N_p - R_A N_A \quad (II)$$

Dividing (I) by plasma volume, V_p , and (II) by the diluting volume of area A , V_A , we have

$$\frac{dc_p}{dt} = (-R_p - R_B) c_p + (R_A - R_B) \frac{V_A}{V_p} c_A + R_B c_0 \quad (III)$$

$$\frac{dc_A}{dt} = R_{pA} \frac{V_p}{V_A} c_p - R_A c_A \quad (IV)$$

Differentiating (III) with regard to t , multiplying (III) by R_A , multiplying (IV) by $(R_A - R_B) \frac{V_A}{V_p}$ and adding these three equations, we have

$$\frac{d^2 c_p}{dt^2} + (R_p + R_A + R_B) \frac{dc_p}{dt} + (R_A R_{pB} + R_A R_B + R_B R_{pA}) c_p - R_A R_B c_0 = 0 \quad (V)$$

Equation (V) is a standard form⁵ of differential equation of the type $\frac{d^2 y}{dt^2} + p_1 \frac{dy}{dt} + p_2 y = p_3$, where the p 's are constants, and has for its solution

$$y - \frac{p_3}{p_2} = a_1 e^{\frac{-p_1 - \sqrt{p_1^2 - 4p_2}}{2} t} + a_2 e^{\frac{-p_1 + \sqrt{p_1^2 - 4p_2}}{2} t} \quad (VI)$$

in which a_1 and a_2 are constants of integration.

Equation (VI) is identical with equation (3) of the text, where

$$b_1 = \frac{-p_1 - \sqrt{p_1^2 - 4p_2}}{2} \quad \text{and} \quad b_2 = \frac{-p_1 + \sqrt{p_1^2 - 4p_2}}{2}$$

Evaluation of a_1 and a_2 from the conditions of concentration and slope at 0 time, and the substitution of the constants of equation (V) for the p 's lead to equations (4) to (7) of the text.

⁵ See, for example, D. A. Murray, *Differential equations*, 1921, p. 70.

REFERENCES

- AMBERSON, W. R., J. FLEXNER, F. R. STEGGERDA, A. G. MULDER, M. J. TENDLER, D. S. PANKRATS AND E. P. LANG. *J. Cell. and Comp. Physiol.* 5: 359, 1934.
- AMBERSON, W. R., T. P. NASH, A. G. MULDER AND D. BINNS. *This Journal* 122: 224, 1938.
- BELL, J. R., A. M. CLARK AND D. P. CUTHBERTSON. *J. Physiol.* 92: 361, 1938.
- BLALOCK, A. *Arch. Surg.* 20: 959, 1930.
- DUNCAN, G. AND A. BLALOCK. *Ann. Surg.* 115: 684, 1942.
- ELLENBERGLER, W. AND H. BAUM. *Systematische und topographische Anatomie des Hundes.* Berlin, 1891.
- FLEXNER, L. B. AND H. A. POHL. *This Journal* 132: 594, 1941.
- FLEXNER, L. B., A. GELLHORN AND M. MERRELL. *J. Biol. Chem.* 144: 35, 1942.
- GREENBERG, D. M., R. B. AIRD, M. D. BOELTER, W. W. CAMPBELL, W. E. COHN AND M. M. MURAYAMA. *This Journal* 140: 47, 1943.
- GREGERSEN, M. E., F. GIBSON AND E. A. STEAD. *This Journal* 113: 54, 1935.
- GREGERSEN, M. E. AND F. D. STEWART. *This Journal* 125: 142, 1939.
- HARKINS, H. N. *Surgery* 9: 231, 447, 607, 1941.
- KROGH, A. *The anatomy and physiology of capillaries.* New Haven, 1922, Yale University Press.
- MANERY, J. F. AND A. B. HASTINGS. *J. Biol. Chem.* 127: 657, 1939.
- MEEK, W. J. *Northwest. Med.* 35: 325, 1936.
- MERRELL, M., A. GELLHORN AND L. B. FLEXNER. *J. Biol. Chem.* 153: 83, 1944.
- MOON, V. H. *Shock and related capillary phenomena.* New York, 1938, Oxford University Press.
- PHEMISTER, D. B. AND L. HANDY. *J. Physiol.* 64: 155, 1927.
- ROOMP, N. W. *Anesth. and Analg.* 17: 237, 1938.
- SMITH, H. P., H. R. ARNOLD AND G. H. WHIPPLE. *This Journal* 56: 336, 1921.
- WALLACE, C. B. AND B. B. BRODIE. *J. Pharmacol. and Exper. Therap.* 65: 214, 1939.

REFLEXES IN THE ANTERIOR TIBIAL MUSCLE AFTER CORD ASPHYXIATION¹

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It has been found previously (6) that myotatic reflexes may recover after periods of cord asphyxiation which abolish the flexion reflex permanently. Thus after 55 and 65 minutes of cord asphyxiation, no flexion reflex could be elicited at any time by pinching the leg, whereas reflex tone and even tendon reflexes could be observed at certain periods after the asphyxiation. For all the observations on myotatic reflexes, extensor muscles have been used (6, 5) whereas the flexion reflex was observed, of course, in flexor muscles. It seemed of interest to investigate the myotatic- and the flexion reflex after cord asphyxiation in the same muscle. Tendon- and stretch reflexes can be elicited in the anterior tibial muscle (13, 1, 2), which is also one of the principal muscles active in the flexion reflex. In the present investigation, tone and the flexion reflex were examined in this muscle after relatively long periods of cord asphyxiation.

METHODS. In a preliminary operation the animal was made spinal by ligating the dura with aseptic precautions, thus severing the spinal cord at Th 10-12. The next day the cord was asphyxiated by forcing Ringer solution into the isolated part of the dural cavity under a pressure higher than the blood pressure (24-26 cm. of mercury) (6). Because a large temperature influence on the effects of cord asphyxiation has been demonstrated (8), the asphyxiation was performed with Ringer solution at 38° while the animal was kept at the same temperature, using the methods described before (8). The experiments were performed under light nembutal narcosis. Cats were used exclusively.

In all experiments one shank of the animal was fixed on a board with screws through the bone. The tendon of the anterior tibial muscle was connected over two trolleys with an isotonic lever writing on a smoked drum. On the heterolateral side the contractions of a different flexor- or of an extensor muscle were recorded simultaneously for comparison. For the recording of the quadriceps or hamstrings on that side, the femur was fixed and the movements of the shank were recorded on the same smoked drum. When the hamstring contractions were recorded, the quadriceps tendon or the femoral nerve was severed; besides the sciatic nerve was transected distal of the branches to the hamstrings excluding the knee-flexion caused by contraction of the gastrocnemius (an extensor muscle). When the contractions of the quadriceps were recorded, the sciatic nerve was severed proximal of the branches to the hamstrings. In some experiments the contractions of the gastrocnemius-soleus muscle were recorded for comparison, fixing the shank with screws and connecting the Achilles tendon with a recording lever.

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Muscle action potentials were recorded with a Matthews' oscillograph, using silver wires placed in the muscle as electrodes.

RESULTS. *Initial and secondary tone.* Two periods of tone, the initial and the secondary tone, have been observed in extensor muscles after asphyxiations of the cord of 55 and 65 minutes (5). The initial tone was always weak as compared with the secondary tone. After 75 minutes of asphyxiation, no spontaneous extensor tone has been observed.

In the anterior tibial muscle, too, both initial and secondary tone were found after cord asphyxiations of 55 and 65 minutes' duration (fig. 1). The initial tone was slight and often consisted of an irregular series of muscle contractions. The secondary tone was quite pronounced in many experiments. Usually irregular muscle contractions were superimposed upon this tonic contraction (fig. 1). No spontaneous tone was found in the anterior tibial muscle after 75 minutes of cord asphyxiation. The time relations of initial and secondary tone after 55 and 65

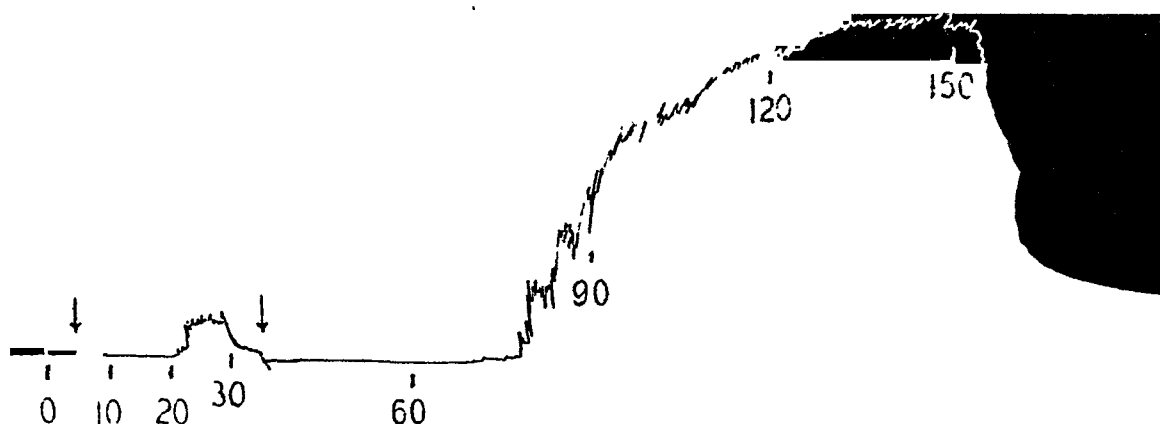


Fig. 1. Initial and secondary tone in the anterior tibial muscle after 55 minutes of cord asphyxiation. The figures in the record show the number of minutes after the end of cord asphyxiation. The arrows indicate artefacts.

minutes' asphyxiation are given in table 1. As this table shows, the initial tone in the majority of the experiments was present between the 20th and 35th minute after the end of asphyxiation. After a toneless interval the secondary tone began 1 to 5 hours after asphyxiation and often disappeared a few hours later. In some experiments it outlasted the period of observation, however. The time relations correspond rather closely with those of the initial and secondary tone found previously in extensor muscles (5). The similarity in the course of initial and secondary tone in flexor and extensor muscles is further demonstrated in experiments 5, 8, 9, 13, 14, 17 and 18, table 1, in which these phenomena were recorded simultaneously in the anterior tibial and in an extensor muscle. The parallelism is not complete, however. The initial tone tends to begin and end slightly later in the anterior tibial muscle, whereas the secondary tone has a tendency to start later in the extensor muscles.

An initial and a secondary tone have also been found in the hamstrings after 55 and 65 minutes of cord asphyxiation. Table 1 shows, in a few experiments (nos.

1, 7 and 10), the time relations of these periods of tone of the hamstrings as compared with those of the anterior tibial muscle in the same animal. The similarity in the course of tone in these two muscles is great. The tone in the hamstrings always was weak in comparison with the corresponding contractions in the anterior tibial muscle.

TABLE 1

Appearance and disappearance of initial and secondary tone after 55 to 65 minutes of cord asphyxiation in the anterior tibial muscle and, in some experiments, in other muscles

NO. OF EXPERIMENT	DURATION OF ASPHYXIATION	MUSCLE	INITIAL TONE		SECONDARY TONE	
			Start	End	Start	End
	<i>min.</i>					
1	65	Anterior tibial			50	300
		Hamstrings			50	300
2	65	Anterior tibial			240	320
3	65	Anterior tibial	20	35		
4	65	Anterior tibial				
5	60	Anterior tibial			80	*
		Quadriceps			110	*
6	55	Anterior tibial	28	35	90	240
7	55	Anterior tibial	30	35	70	*
		Hamstrings			80	*
8	55	Anterior tibial	8	22		
		Gastro-soleus	6	16		
9	55	Anterior tibial	12	26	50	*
		Gastro-soleus	10	24	60	*
10	55	Anterior tibial	18	30	65	200
		Hamstrings	16	30	70	190
11	55	Anterior tibial	20	30	90	160
12	55	Anterior tibial				
13	55	Anterior tibial	20	25		
		Quadriceps	15	25		
14	55	Anterior tibial			85	270
		Quadriceps			100	240
15	55	Anterior tibial				
16	55	Anterior tibial	40	50	290	*
17	55	Anterior tibial	20	30	75	160
		Quadriceps	15	25	90	200
18	55	Anterior tibial	20	30	65	360
		Quadriceps	15	30	70	270

The figures in the last four columns show the time in minutes after the end of cord asphyxiation of beginning and end of initial and secondary tone. An asterisk in the last column indicates that the secondary tone was still present at the end of the period of observation.

It has been shown previously (5) that tone in the extensor muscles could be elicited during the toneless interval, between initial and secondary tone, by a renewed asphyxiation of the cord. The same phenomenon was observed in the anterior tibial muscle as well as in the hamstrings.

The nature of the tone in the anterior tibial muscle. It has been shown previously

(5) that action potentials led off from an extensor muscle during initial and secondary tone increased when the muscle was stretched, and decreased when it was relaxed, indicating that these contractions are reflex in nature.

It was possible to demonstrate the reflex nature of the secondary tone in the anterior tibial muscle in the same way. Figure 2 shows the effects of stretch and relaxation of the muscle on the action potentials of the anterior tibial muscle 270 minutes after the end of a 55 minute cord asphyxiation. The increase in action potentials on stretch, and decrease on relaxation, indicate that the tone is elicited reflexly by stimulation of the stretch receptors in the muscle. The effects of stretch and relaxation were not always as pronounced as in the experiment shown in figure 2; especially when the electrical activity was high, the effect was small or even absent. It is certain, however, that the secondary tone in the anterior tibial muscle has a reflex component.

The initial tone in the anterior tibial muscle usually was so weak and fleeting that it was not possible to obtain similar evidence for this period of tone.

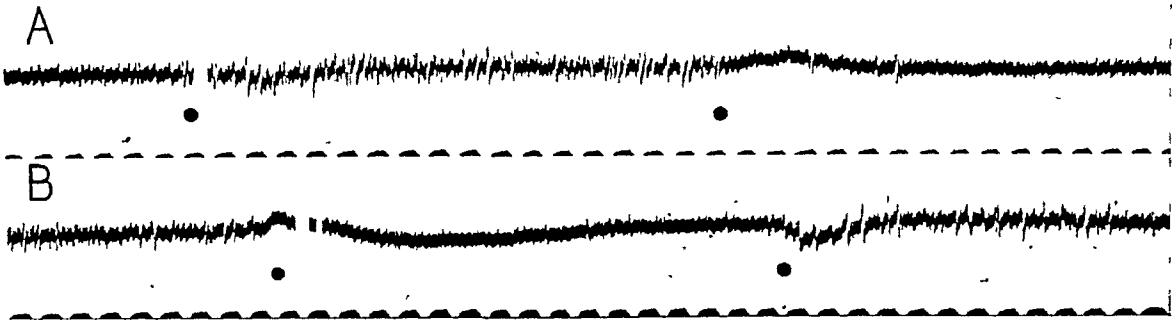


Fig. 2. Action potentials of the anterior tibial muscle taken about $4\frac{1}{2}$ hours after the end of a 55 minute cord asphyxiation. The muscle had shown an initial tone, and the secondary tone had set in 75 minutes after the end of the asphyxiation. A—The anterior tibial muscle is stretched during the period between the two black dots. B—The muscle is relaxed between the black dots.

Flexion reflex. It has not been possible to elicit a flexion reflex at any time after a 55 or 65 minutes cord asphyxiation by pinching the leg. Even when the anterior tibial contractions were recorded with a sensitive lever system, this stimulus was ineffective.

The central stump of the tibial nerve was stimulated with strong faradic stimuli in a number of cases. In none of the experiments has this stimulus elicited, during the initial tone, muscle contractions which could be distinguished from the spontaneous, slight, irregular contractions usually present in the muscle during this period of tone. In the interval between the periods of tone, the stimulation of the tibial nerve had no effect. During the first development of the secondary tone, stimulation of this nerve caused, in some experiments, slight anterior tibial contractions which were unmistakably related to the stimulus. During the maximum of secondary tone there were again present spontaneous irregular muscle contractions (fig. 1) which masked eventual slight responses to the faradization of the tibial nerve. In only one experiment was a response recorded large

enough to be recognized as such. It thus can be concluded that after 55 minutes of cord asphyxiation traces of the flexion reflex can be demonstrated only very occasionally, when sensitive recording devices are used and massive stimuli are given.

In those experiments in which the contractions of the anterior tibial and quadriceps muscle were recorded simultaneously, no trace of crossed extension was found during the stimulation of the heterolateral tibial nerve.

DISCUSSION. Lloyd (9, 10, 11) recently found that the myotatic reflex is a monosynaptic, the flexion reflex a multisynaptic reflex. In other words, the reflex arc of the myotatic reflexes consists of only two neurons, the sensory and the motor neuron, whereas in the arc of the flexion reflex, these neurons are connected by at least one internuncial neuron. Lloyd based this conclusion on a study of the reduced reflex time which allows in the myotatic reflexes for only one, in the flexion reflex for more than one synaptic delay. He demonstrated the monosynaptic myotatic reflex not only in the extensor muscles but also in the anterior tibial muscle.

The study of the action potentials of the secondary tone after 55 minutes of cord asphyxiation has shown the presence of a myotatic reflex in the anterior tibial muscle which, according to the above viewpoint, would be monosynaptic. The traces of anterior tibial contraction caused occasionally by the faradization of the tibial nerve are probably the last rests of the multisynaptic flexion reflex, since Lloyd found that no monosynaptic reflex could be elicited in the peroneal nerve by stimulation of the tibial nerve. It thus can be concluded that the multisynaptic flexion reflex is more severely damaged by cord asphyxiation than the monosynaptic myotatic reflexes.

Since the initial and secondary tone appeared in both the anterior tibial and extensor muscles with almost the same time relations after asphyxiation periods of 55 and 65 minutes, but remained absent after asphyxiations of 75 minutes' duration, it can be concluded that the extensor and flexor motor neurons are about equally sensitive to asphyxiation.

It has been found previously (3) that even after 65 minutes of cord asphyxiation by increased pressure in the dural cavity, no degeneration was produced in the dorsal roots after an adequate waiting period, whereas a marked degeneration was present in the anterior roots. This is perhaps because the spinal ganglia are situated outside the pressure area. The reflex path of the myotatic reflex up to the motor neurons thus has the potentiality of a speedy functional recovery, assuming that the parts of the sensory neuron situated inside the spinal cord are not more easily damaged by asphyxiation than the dorsal root fibers. The perikarya are probably the structures damaged by asphyxiation (7), and the behavior of the monosynaptic reflexes after asphyxiation thus will depend mainly on the damage incurred by the motor cells.

The myotatic reflex and the flexion reflex in the anterior tibial muscle have the same final common path. The development of a pronounced secondary tone after 55 and 65 minutes of asphyxiation indicates that this final common path is functional, even if it can hardly, or not at all, be utilized in the flexion reflex.

The difference between the myotatic and the flexion reflex is the presence of internuncial neurons in the reflex arcs of the latter reflex, and a greater sensitivity of the internuncial neurons to asphyxiation would explain the greater vulnerability of that reflex. However, the fact that two neurons of the flexion reflex are situated in the asphyxiated area, whereas only one neuron of the myotatic reflex is subjected to asphyxiation, may in itself be a cause for the greater depression of the flexion reflex. The motor neurons which become functional again after asphyxiation, and which may constitute only a small part of the total pool, can all be used in the myotatic reflex since the sensory neuron is not affected by asphyxiation. In the flexion reflex not only part of the motor neurons, but also part of the internuncials will remain inactive. This will decrease the number of impulses reaching a motor neuron within the period in which they can reinforce each other (12) and thus will decrease spatial summation. Furthermore, it is conceivable that part of the motor neurons which have recovered cannot be activated in the flexion reflex because the internuncials, through which the impulse must pass, did not recover. Also, the impulses which have passed through recovered internuncials, may end up in inactive motor neurons. Thus, the longer the chain of neurons the impulse has to pass, the smaller the chance that an active motor neuron will be reached. It must be understood that the distribution of the impulse from one internuncial over many motor neurons will counteract this effect. Nevertheless, it may be responsible for the ease with which crossed extension is abolished by asphyxiation (8), since this heterolateral reflex probably has more than one internuncial neuron in its arc.

These considerations suggest that in general the more complicated central nervous mechanisms will be more vulnerable to asphyxiation due to the circumstance that longer neuron chains are involved in their functioning.

I am indebted to Mrs. J. Wiersma for her valuable assistance.

SUMMARY

1. The same periods of tone described for the extensor muscles have been found in the anterior tibial muscle after 55 and 65 minutes of asphyxiation of the spinal cord.

2. Evidence for the reflex nature of certain phases of this tone in the anterior tibial muscle has been found.

3. Only occasional traces of the flexion reflex have been demonstrated after cord asphyxiation of these durations, though the presence of tone in the anterior tibial muscle is an indication that the final common path of both reflexes is functioning.

4. An explanation for the difference in vulnerability of flexion- and myotatic reflexes to cord asphyxiation is sought in the difference in the number of neurons forming the reflex arcs of these reflexes.

REFERENCES

- (1) ASHYMA, C. *Quart. J. exper. Physiol.* 9: 265, 1915.
- (2) DENNY-BROWN, D. E. *Proc. Roy. Soc.* 104B: 252, 1929.

- (3) HARREVELD, A. VAN. This Journal 131: 1, 1940.
- (4) HARREVELD, A. VAN. This Journal 133: 572, 1941.
- (5) HARREVELD, A. VAN. This Journal 139: 617, 1943.
- (6) HARREVELD, A. VAN AND G. MARMONT. J. Neurophysiol. 2: 101, 1939.
- (7) HARREVELD, A. VAN AND D. B. TYLER. This Journal 138: 140, 1942.
- (8) HARREVELD, A. VAN AND D. B. TYLER. This Journal 142: 32, 1944.
- (9) LLOYD, D. P. C. J. Neurophysiol. 6: 111, 1943.
- (10) LLOYD, D. P. C. J. Neurophysiol. 6: 293, 1943.
- (11) LLOYD, D. P. C. J. Neurophysiol. 6: 317, 1943.
- (12) LORENTE DE NO, R. This Journal 113: 524, 1935.
- (13) SHERRINGTON, C. S. Schäfer's Textbook of Physiology 2: 1002, 1900.

INDUCED VARIATIONS IN CELL FLUID VOLUME IN THE STUDY OF SHIFTS OF BODY FLUID

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In problems that relate to general disturbances of the body fluids as well as in the general study of cell physiology, the question of mobility of, or variations in, the cell fluids frequently arises. The prevailing opinion as to the behavior of cell fluids is expressed by Peters (1) as follows: that cells adjust themselves to variations in osmotic pressure of the fluid environment by interchanges of fluid, rather than salts. This adjustment would be followed by changes in the cell volume, and by variation in the total cellular fluid of the body. This supposition has been supported by observations made by Gilman (2), who injected 5 per cent dextrose intraperitoneally in dogs, producing a diffusion of sodium into this fluid. This in turn, causing a reduction of osmotic pressure in the extracellular fluid, was followed by an adjustment mechanism consisting of passage of water into the cells. These changes were calculated by sodium determinations. Hemoconcentration occurred in these animals to a remarkable degree. Similar results were obtained by Darrow and Yannet (3), and we (4), also have observed some calculated increase in cellular fluids, as well as hemoconcentration in dogs given 5 per cent dextrose intraperitoneally. In this type of study some difficulty of interpretation arises from the fact that there occurs marked local accumulations of edema fluid in the connective tissue outside the peritoneal lining. We have observed this to be true in all animals receiving 5 per cent glucose intraperitoneally. A local extravascular fluid accumulation might account for some of the hemoconcentration and therefore this phenomenon may not be due entirely to the intracellular accumulation of water. Furthermore equilibration of ions in large local fluid collections is known to occur slowly (5) and, since the volume of this subperitoneal edema is not subject to measurement, correction cannot be made accordingly. It is therefore possible that some interference in calculation of intracellular and extracellular fluids from changes in plasma sodium might be introduced by this factor. Another theoretical objection to the conclusion that fluid freely enters cells in the experiment described is the probable expected behavior of the glucose ion. Although temporary equilibration of osmotic pressure differences might be occasioned by fluid redistribution, subsequently glucose would be expected to enter the cells, restoring the osmotic equilibrium and bringing about a return of fluid volume to the extracellular phase. Whether this process occurs rapidly enough and before the glucose is metabolized, to have an influence on the distribution of fluid in and outside the cells, is open to question. These objections do not seem to be sufficient to question seriously the results as obtained by Gilman, and at the present time there is a strong inclination to ac-

cept his conclusions that cell fluid volume is changeable according to the osmotic pressure differences that may arise between cells and extracellular fluids.

Other evidence that changes in fluid volume of the body cells can and do take place is afforded by the studies on muscle tissue of dogs. Hastings and Eichelberger (6) found that increase of osmotic pressure in the extracellular fluid of the muscle led to a decrease of cell water in muscle, and a decrease in the extracellular osmotic pressure produced an increase in the cell fluid content. Changes in pH also caused changes in fluid content of muscle cells. In dogs receiving hypertonic plasma, small shifts of fluid from intracellular to extracellular phases have been shown to occur (7).

Aside from these observations on cell fluid changes made by Gilman, Darrow and Yannet, and Hastings and Eichelberger, evidence relating to fluid shifts of cells is obtained from erythrocytes, various plant cells, and small unicellular organisms. These latter studies cannot, in our present state of knowledge, be taken as evidence relating to tissue cells.

The question of mobility of cellular fluids is not without considerable importance in medical problems. Correlation of any possible variation in cell fluid volume with morphological changes has not been carried out in any detail. Whether the process spoken of as intracellular edema, or acute parenchymatous degeneration is actually, fundamentally, a matter of disturbance of water balance in the cells is an intriguing possibility. In infections, fever from other causes, anoxemia, and chemical poisonings where cells are said to become the seat of swelling due to increase in water content, it is possible that the mechanism is that of some generalized disturbance of osmotic equilibrium between cells and extracellular fluids. Aside from the fact that such increases in cellular fluid might modify cell metabolism, it is also likely that depletion of the extracellular fluid might be carried to a significant degree. Such fluid shifts, if understood to occur, and also by what means, might be corrected by simple therapeutic procedures, assuming that the change itself is deleterious to the cell function. Loss of cell fluids is a problem likewise of great significance only briefly studied as to its occurrence and significance (8). In the problems relating to depletion of blood volume with subsequent replenishment from extravascular fluids, the part played by cellular fluids is an incompletely known but possibly significant one. These and other aspects of the cell fluid problem indicate the significance of an understanding of the interchange of cellular fluids with extracellular fluids.

The approaches to this study are difficult. Study of the responses of cells may be made with single cells, as erythrocytes, *Arbacia* eggs, etc. These have the disadvantage of representing special circumstances that cannot be directly transferred to tissue cells in general. Or the study may be confined to observations on single tissues where fluid and electrolyte studies are made. This form of investigation has much to its credit but whether other cells in the body behave in a manner identical, for example to muscle cells, cannot be stated from this localized type of investigation.

We have chosen, instead of these two methods of study, the aspect of total cellular and extracellular fluid volumes, as representing the most acute form of

approach to this question. It is felt that the total cellular and extracellular fluid volume changes under varying conditions would represent the most important behavior of the body fluids.

METHODS. In the present study, then, an attempt has been made to produce and measure shifts of fluid from extracellular to intracellular phase and in the opposite direction also, the objective in mind being only that of demonstrating that such shifts can occur.

Healthy adult mongrel dogs were utilized in this study. Smaller animals are unsuitable since variations of fluid volume changes probably cannot be ascertained with the required degree of accuracy.

Six dogs were given large intravenous injections of hypotonic, 0.45 per cent, saline (dog 12 received 0.22 per cent). These injections were given into the jugular veins in animals anesthetized with pentobarbital-sodium. An average of 1330 cc. was given to each animal over a period of about 2 hours. The purpose of this series was to induce fluid to enter the cells as a result of reduction of the extracellular fluid osmotic pressure. The ureters of these dogs were ligated in order to prevent the kidneys from excreting water and thus avoiding the osmotic pressure change.

In eight dogs, 50 per cent sucrose was given intravenously with conditions as indicated in the animals receiving hypotonic saline except that the ureters were not ligated. Instead, a catheter was placed suprapubically in the bladder so that urine could be accurately collected. Dogs 1 and 2 received 50 cc. of the hypertonic sucrose, while the remaining dogs received approximately 200 cc. Those animals receiving 200 cc. were given injections of 50 cc. each over a period of about 1 hour.

Measurements of the extracellular fluids were made by two methods. The first procedure was that of determining the amount of fluid available for solution of thiocyanate (9). The volume of fluid in the body available for solution of thiocyanate was accepted as representing predominantly the extracellular fluid, although it is likely that a small amount of cellular fluid is included in the calculation due to the thiocyanate ion slowly entering cells (4). A control determination of fluid available for solution of thiocyanate was made in each case, and about one hour after completion of the injection a second determination was made by reinjecting thiocyanate. 150 to 200 mgm. of sodium thiocyanate were injected for each determination. The method of Laviates and others (10), modified for the Lumetron photoelectric colorimeter, was used for the determination of thiocyanate.

The second method utilized in calculating change in extracellular fluid was that of determination of the plasma sodium before and following the intravenous injection. The method of Butler and Tuthill (11) was used for the sodium determinations. The assumption was made that sodium ions do not enter the cells, hence changes in fluid volume of the extracellular space were considered to be reflected by changes in sodium concentration of the plasma (extracellular fluid). In the calculation employed, account was taken of sodium added to the extracellular fluids in the injected solution, and also sodium lost in the urine.

In these calculations, the original extracellular fluid was that indicated by the control thiocyanate procedure.

Determination of cell fluid changes was indirect. No accepted method for directly measuring cellular fluid being available, changes were calculated from urine output, volume of fluid administered, and variations in extracellular fluid. Animals were weighed before and at the end of the experiment and it was found that no significant amount (less than 30 grams) of fluid was lost by other means during the experiment. Total body fluids were arbitrarily assumed to be 75 per cent of the body weight. It was further assumed that the total body fluid remained constant over the experimental period, and that any change in extracellular fluid volume, unaccounted for by urine output of fluid administered would be accompanied by a complementary change in the cellular fluid. Although these assumptions are not above question they are reasonable. The calculation of cell fluid was as follows:

$$C_1 = T - Ex_1$$

$$C_2 = C_1 + (Ex_1 - Ex_2) + F - U$$

C_1 = Control cell fluid

C_2 = Second cell fluid

T = Total body fluid (75 per cent of body weight)

Ex_1 = Control extracellular fluid (fluid available for thiocyanate)

Ex_2 = 2nd. Extracellular fluid (by thiocyanate or sodium)

F = Volume of fluid administered

U = Urine volume

In an additional seven dogs, measurements of extracellular and cellular fluids were made under control conditions consisting of nembutal anesthesia alone without injecting any fluids.

RESULTS AND DISCUSSION. The results of the entire experiment are expressed in table 1. In the series of control experiments the extracellular fluid remained fairly constant and sodium and thiocyanate methods checked well in indicating these relatively slight changes. Over the period of several hours of observation the average change in extracellular fluid was 0.3 per cent by the thiocyanate method and 1.4 per cent by the sodium method. The maximum variation was 5.5 per cent. These results are in accord with previous observations (4). The question that arises here is whether those few variations of larger magnitude, though comparatively small, actually occurred, or whether they are indicative of error of the method. The fact that sodium and thiocyanate methods revealed a similar change would be evidence that some increase actually did occur in the extracellular fluid. In this group of control animals cellular fluid changes were of the same order as the changes in extracellular fluid, but in the opposite direction. The percentage change was slightly less due to the larger size of the cellular phase.

An analysis of these figures in the control group shows the mean change in intracellular fluid to be -30 cc. The standard deviation is 25 cc.

In the group of dogs receiving hypotonic saline the expectation of finding an increase in extracellular fluid was fulfilled. The thiocyanate and sodium figures

TABLE 1
Demonstrates changes in extracellular and intracellular fluids in all experiments

DOG NO.	WEIGHT	SCN AVAILABLE FLUID- CONTROL	CHANGE IN EXTRA- CELLULAR FLUID*	PER CENT CHANGE	CELLULAR FLUID- CONTROL	CHANGE IN CELLULAR FLUID*	PER CENT CHANGE	REMARKS
1	10.65	3030	-90 +50	3.0 1.7	4920	+90 -50	1.8 1.0	Control
2	10.65	3370	+40 +20	1.2 0.6	4580	-40 -20	0.9 0.4	Control
3	13.10	3790	+210 +150	5.5 4.4	6030	-210 -150	3.5 2.5	Control
4	10.80	4080	0 0	0 0	4020	0 0	0 0	Control
5	11.07	3360	+90 +100	2.7 3.0	4960	-90 -100	1.8 2.0	Control
6	9.57	3020	-20 -20	0.7 0.7	4180	+20 +20	0.5 0.5	Control
7	10.10	3220	-150 +40	4.7 1.2	4350	+150 -40	3.5 0.9	Control
Average...	10.85	3410	+10 +50	0.3 1.4	4720	-10 -50	0.2 1.1	Control
Average both methods..			+30			-30		
8	10.70	4160	+840 +970	20.5 23.7	3860	+620 +490	16.0 12.5	1460 cc. 0.45% NaCl
9	12.60	5000	+770	15.4	4470	+570	12.6	1340 cc. 0.45% NaCl
10	8.40	2370	+890 +850	37.1 35.4	3930	+170 +210	4.3 7.2	1060 cc. 0.45% NaCl
11	9.09	2660	+1340 +1340	50.0 50.0	4160	+350 +350	8.3 8.3	1690 cc. 0.45% NaCl
12	10.65	2860	+590 +700	20.3 24.1	5090	+540 +430	10.6 8.4	1130 cc. 0.22% NaCl
13	8.14	2600	+600	25.4	3470	+720	20.6	1380 cc. 0.45% NaCl
Average..	9.93	3310	+890 +900	26.0 27.3	4165	+450 +440	10.5 10.7	1330 cc.
Average both methods..			+895			+445		
14**	12.52	4170	-70	1.7	5200	-70	1.3	50 cc. 50% Sucrose
15**	11.55	3850	-240 -210	6.3 5.5	4770	+130 +100	2.7 2.1	50 cc. 50% Sucrose
16	8.14	3050	-340	11.3	3020	-210	7.0	200 cc. 50% Sucrose
17	11.17	4030	-420 -460	10.5 11.5	4370	-220 -180	5.0 4.1	190 cc. 50% Sucrose
18	6.10	2110	-260 -170	12.4 8.1	2460	-320 -410	12.8 16.4	190 cc. 50% Sucrose
19	6.72	2540	-550 -430	22.4 17.2	2480	-20 -150	0.8 6.0	150 cc. 50% Sucrose
20	5.94	2110	-210 -300	10.0 14.3	2310	-350 -260	15.2 11.3	150 cc. 50% Sucrose
21	10.07	3490	-190	5.4	4080	-400	10.0	190 cc. 50% Sucrose
Average..	8.02	2890	-325 -340	11.3 11.5	3120	-260 -240	8.4 7.8	180 cc. 50% Sucrose
Average both methods..			-335			-250		

* Figures in upper portions of brackets are representative of the thiocyanate method, and those in the lower portions the sodium method.

** Dogs 14 and 15 received only 50 cc. of hypertonic sucrose, and are not included in the calculation of the averages.

checked well. The average increase entailed by injection of an average of 1330 cc. was 890 cc. by the thiocyanate method and 900 cc. by the sodium method. The average increase of intracellular fluid was 450 cc. and 440 cc. respectively. This was an average increase of 10.5 and 10.7 per cent over the original cellular fluid.

For statistical purposes, the results obtained by the sodium method and thiocyanate method are combined in the calculations. The mean increase of cellular fluid is 445 cc. in the animals receiving hypotonic saline. Standard deviation is 54 cc. The difference between the mean of the control group and the mean of the hypotonic saline group is 415 cc., which is 22.6 times the standard error of the mean difference, 18.4 cc. Thus the difference between the observed means in the two groups is significant (12). These data therefore constitute satisfactory evidence that the cells of the body are capable of adjusting themselves by changes in volume as a result of diffusion of water.

An evaluation of the factors concerned in this redistribution of fluids is attended by considerable difficulty. Cell membrane permeability to water, impermeability to sodium, and some elasticity of the cell membrane are inferred to exist in order for these circumstances to be fulfilled. The factor which cannot be evaluated in our present state of knowledge is what the limits of distensibility of the cell membrane, and thus its hydrostatic pressure effect, would be. This factor would be the one which would determine the limit of accommodation to osmotic pressure differences between the cell and its environment. Distensibility of organ capsules would also possibly be a contributory factor in the process of intracellular diffusion of water.

The first two animals which were given hypertonic sucrose received such small injections that the changes produced, if any, were not measurable. These two animals probably should not, therefore, be considered in an analysis of the figures presented here. An analysis of the data in this group of animals, combining the results of the sodium and thiocyanate methods to determine the number of observations made, reveals the mean decrease in cellular fluid to be 250 cc. with a standard deviation of 40 cc. The difference between the mean of this sucrose group and the control group is 220 cc. This figure is 15.4 times the standard error of the mean difference which is 14.3 cc.

Greater decreases were found to occur in the extracellular fluid where average decreases of 11.3 per cent by the thiocyanate method and 11.5 per cent by the sodium method were encountered. The decrease of cellular and extracellular fluids was accounted for entirely on the basis of the large amount of urine output which averaged 730 cc. for the group of six dogs.

It is believed that these figures are indicative of the fact that the body cells are capable of giving up water to the extracellular space when the osmotic pressure of the latter is increased. In order for this to occur it is necessary to postulate that sucrose remains in the extracellular space sufficiently long to exert its osmotic effect and that it does not enter cells. This latter has been shown to be true by Laviertes and others (10). It is also necessary to postulate permeability of the cell membrane to water, and that the cell is capable of contraction in size.

Cells would be expected to undergo decrease in size without difficulty. In withdrawing water from cells by increasing osmotic pressure in the extracellular space the matter of cell membrane distensibility would not play the significant rôle which it might play in increase in cell size. It might even be possible to calculate cell volume changes on the basis of extracellular osmotic pressure increase. In order that this be done in our experiments it would be necessary to have available information in regard to the amount of sucrose that was excreted in the urine. Unfortunately that information is not available.

If it is thus possible to show that cells can increase or decrease in size due to water shifts as a result of changes in osmotic pressure of extracellular fluid, it is quite probable that similar shifts can occur as a result of modification of osmotic pressure within the cells. To what extent such a mechanism might operate in health or disease has not been determined. Nor has it been shown that variations of osmotic pressure of the extracellular fluid with consequent shifts of cellular fluid occur to any significant degree during health or disease. The fact that changes can be induced under experimental condition only suggests that it might occur spontaneously. Studies of extracellular fluids have led to the belief, which is in accord with the findings in this study, that the interstitial fluid is a sort of buffer reservoir of fluid that is available to give or receive fluid from cells or plasma as occasions demand through osmotic pressure changes. And yet an increase or decrease in volume of interstitial fluid may be accompanied by a similar but lesser change in the plasma volume. Since sodium and chloride are confined to the extracellular fluid, cellular fluids would not be expected to participate in simple increases in volume of the extracellular compartment without accompanying changes in osmotic pressure.

After preparation of this paper, recent work on dehydration by Elkington and others (13, 14, 15), came to our attention. In their work on experimental hypertonicity they were able to produce definite decreases of cellular fluid by intravenous and intraperitoneal injections of hypertonic sodium chloride solutions. Reductions of as much as 16 to 20 per cent of the intracellular volume were accomplished in some animals. The authors discuss the significance of cell dehydration and other aspects of the problem of cellular and extracellular fluids.

SUMMARY

As determined by alterations in the amount of fluid available for solution of thiocyanate and in the extracellular fluid volume calculated by changes in plasma sodium, variations in the volume of total cellular fluids were produced in dogs. Introduction of hypotonic saline into the extracellular fluids produced a decrease in osmotic pressure in the extracellular fluid with a consequent increase in cellular fluid volume. Hypertonic sucrose caused the opposite change, namely, an increase of extracellular osmotic pressure, and a decrease of cellular fluid.

In order for these variations in cell fluids to occur it is necessary to postulate that the cell membrane is practically impermeable to sodium, chloride, sucrose, that it is freely permeable to water, that the cell membrane is susceptible to some degree of distention, and that cells can contract in volume.

Extracellular fluids respond in a manner directly opposed to the cell fluid changes as long as regulatory excretory mechanisms are not in full operation.

It is concluded that cells do adjust themselves to changes in osmotic pressure of their environmental fluid by fluid shifts rather than electrolyte shifts, providing the electrolytes concerned do not readily penetrate the cell membrane.

Assistance was given in some experiments by Mr. C. E. Gordon and Mr. W. W. Plasek.

REFERENCES

- (1) PETERS, J. P. Body water. Charles C. Thomas, Springfield, Ill. 1935.
- (2) GILMAN, A. This Journal 108: 662, 1934.
- (3) DARROW, D. C. AND H. YANNET. J. Clin. Investigation 14: 266, 1935.
- (4) ASHWORTH, C. T., E. E. MUIRHEAD, O. F. THOMAS AND J. M. HILL. This Journal 139: 255, 1943.
- (5) GILLIGAN, D. R. AND M. D. ALTSCHULE. J. Clin. Investigation 14: 659, 1939.
- (6) HASTINGS, A. B. AND L. EICHELBERGER. J. Biol. Chem. 29: 41, 1935 (Proc.).
- (7) ASHWORTH, C. T., E. E. MUIRHEAD AND J. M. HILL. This Journal 136: 194, 1942.
- (8) BUTLER, A. M., C. F. MCKHANN AND J. L. GAMBLE. J. Pediatrics 3: 84, 1933.
- (9) CRANDALL, L. A., JR. AND M. K. ANDERSON. Am. J. Digest. Dis. and Nutrition 1: 126, 1934.
- (10) LAVIETES, P. H., J. BOURDILLON AND K. A. KLINGHOFFER. J. Clin. Investigation 15: 261, 1936.
- (11) BUTLER, A. M. AND E. TUTHILL. J. Biol. Chem. 93: 171, 1931.
- (12) HILL, B. Principles of medical statistics. The Lancet Limited, London, 1942.
- (13) ELKINGTON, J. R. AND A. W. WINKLER. J. Clin. Investigation 23: 93, 1944.
- (14) WINKLER, A. W., J. R. ELKINGTON, J. HOPPER, JR. AND H. E. HOFF. J. Clin. Investigation 23: 103, 1944.
- (15) HOPPER, J., JR., J. R. ELKINGTON AND A. W. WINKLER. J. Clin. Investigation 23: 111, 1944.

HISTOCHEMICAL CHANGES IN THE KIDNEY DURING DIURESIS AND DEHYDRATION

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The majority of experiments concerning the physiology of the normal kidney have dealt with the passage or absorption of various substances through the different anatomical units of the nephron. Another approach to this subject is to attempt to analyze what occurs within the renal tissue itself, particularly in reference to the changes in its intracellular and extracellular components. It is now quite widely accepted that in muscle tissue the chloride is present exclusively in the extracellular fluid while the potassium is mainly intracellular (1). However, these simple relationships cannot be assumed *a priori* to be characteristic of kidney tissue. Eichelberger and Bibler in a study of normal and hydronephrotic kidneys of dogs found that renal tissue, as compared with muscle, contained higher amounts of water, sodium and chloride and a smaller amount of potassium. These differences were especially marked in the medullary portion of the kidney and it was suggested that the excess sodium and chloride might be present in the tubules or the tubular cells (2). Such an interpretation is consistent with the effort of the kidney to conserve these elements.

The experiments to be reported below have had for their purpose an evaluation by chemical means of the relative amounts of the intracellular and extracellular components in the cortical and medullary portions of the rat kidney. These observations have been made both under normal conditions and under conditions of physiological stress imposed by fluid restriction and fluid excess.

It may be stated at once that it has been found that this histochemical approach to the state of the kidney tissue has permitted a quantitative estimate of the relative changes in the intracellular and extracellular phases during diuresis and dehydration.

METHODS. *Physiological technique.* The twenty-four rats used for these analyses were divided into four experimental groups to be referred to as: 1, normal; 2, dehydrated; 3, isotonic saline diuresis, and 4, distilled water diuresis. There were six animals in each group. The normal rats were allowed free access to food and water; the dehydrated rats were given food but no water for periods varying from twenty-four to forty-eight hours; the isotonic saline group received 12 to 24 cc. of isotonic saline (154 mEq. NaCl per liter) intraperitoneally, 6 to 12 cc. subcutaneously, and 7 to 41 cc. by stomach tube over a two to four hour period; and the distilled water diuresis group received 28 to 60 cc. of distilled water by stomach tube over a seven hour period.

Collection and analysis of urine. All rats were isolated in cages and the urinary output was collected under oil by means of a funnel and its volume measured. The specific gravity of the urine was determined by the gradient tube method

(3, 4). Chloride and potassium determinations were made on the urine of about half of the rats. All chloride analyses were done by a Volhard titration and the potassium was determined colorimetrically after precipitation as the chloroplatinate (5). The urine solids were calculated by multiplying the last two numbers of the specific gravity by Long's coefficient (2.66)(6). The urine output, specific gravity, urine solids, and chloride and potassium contents are shown in table 1.

The contrast between the urine of the dehydrated group and the water diuresis group is clearly apparent. The former had a lower output of urine, but higher specific gravity, solid content and concentrations of chloride and potassium than the latter group. The urine of the rats receiving excess isotonic saline contained chloride in approximately the same concentration as it was administered. These differences in the urine of the experimental rats from that of the normal series illustrate the physiological responses of the kidney to the imposed stresses of water deprivation and water excess. If these compensatory mechanisms of the kidney are adequate, the partitioning of the body water in the tissues of the body will be undisturbed; if they are not, changes should be observed in the propor-

TABLE 1

Urine

Average values for the urine output (cc./hr.) on basis of last measured period before death of rat, specific gravity of collected urine, solids (gram/L), chloride content (mEq./L urine), and potassium content (mEq./L urine). Number of animals indicated in parentheses.

	OUTPUT	SP. GRAV.	SOLIDS	CHLORIDE	POTASSIUM
Normal group.. . . .	0.48 (4)	1.040 (6)	106. (6)	89.1 (2)	88.6 (2)
Dehydrated group....	0.13 (3)	1.057 (5)	160. (5)	136.0 (2)	114.7 (1)
Distilled water diuresis group.....	8.45 (4)	1.013 (6)	35. (6)	26.7 (3)	4.30 (3)
Isotonic saline diuresis group.....	11.75 (4)	1.026 (6)	69. (6)	164.9 (3)	6.47 (3)

tions and composition of the extracellular and intracellular phases of the tissues of the body.

Analyses of serum, muscle and kidney were carried out to illustrate this point.

Collection and analysis of the blood and serum. The rats were killed by a blow on the head and the renal vessels were immediately clamped. Blood was obtained from the heart by direct puncture. The density of the serum was estimated by the gradient tube method and the protein content calculated according to the formula of Moore and Van Slyke (7). By assuming 15 grams of non-protein solids per liter of serum, it is then possible to calculate the water content of the serum (5). The chloride content of the serum was determined by a Volhard titration. The serum water and chloride concentration are given in table 2.

The changes observed were in the direction which one would have expected as the result of water deprivation and of excess water and salt solution.

Treatment and analysis of muscle tissue. Approximately one gram samples of thigh muscle were removed and analyzed for total solids, lipide, and chloride by

means of previously described methods (5). In table 3 are shown the lipide-free solids and chloride content of the muscle.

As would be expected, the solid content increased slightly during dehydration and decreased greatly during diuresis, while the chloride concentration increased markedly in the group receiving isotonic saline. The interpretation of these results in terms of the changes occurring in the extracellular and intracellular phases of the muscle will be presented in a later section.

Treatment and analysis of kidney tissue. The kidneys were removed and after being chilled to 5° for 30 minutes, the capsules were stripped. Sections of kidney were taken from one-half of the rats for microscopical study. The kidneys were divided as nearly as possible into cortical and medullary tissue, minced, then each was separated into two fractions comprising two-thirds and one-third of the material, respectively. All of these operations were carried out on hardened filter paper in the cold room (5°C.).

TABLE 2

Serum

Average values for the serum water content (gram/L), and the serum chloride (mEq. Cl/kgm. serum water). Number of animals indicated by parentheses.

	SERUM WATER	SERUM CHLORIDE
Normal group.....	935.0 (6)	111.9 (5)
Dehydrated group..	931.0 (5)	114.1 (4)
Distilled water diu- resis group.....	945.1 (6)	93.4 (5)
Isotonic saline diu- resis group.....	949.2 (5)	119.7 (5)

TABLE 3

Muscle

Average values for the lipide-free solids (gram/kgm.) and chloride content (mEq. Cl/kgm. lipide-free tissue). Number of animals indicated in parentheses.

	SOLIDS	CHLORIDE
Normal group.....	223.4 (5)	14.29 (4)
Dehydrated group..	229.4 (5)	11.98 (5)
Distilled water diu- resis group.....	205.3 (6)	13.43 (6)
Isotonic saline diu- resis group.....	214.0 (5)	22.52 (5)

The one-third portions were placed in tared test tubes, weighed, and dried in the oven at 90°C. for eighteen hours to determine the total solid content.

To the weighed two-thirds portions were added 0.75 N HNO₃ (10.00 cc. to the cortical portions and 2.00 cc. to the medullary portions). The nitric acid extracts were allowed to stand for one hour with frequent stirring, then centrifuged and the liquid decanted off to be used for chloride and potassium analyses. The tissue remaining after extraction was washed with 8 cc. of 10 per cent trichloroacetic acid for 20 minutes, centrifuged and the liquid discarded. Absolute alcohol (8 cc.) was added and allowed to stand for 12 hours. This was decanted off and followed by an equal amount of alcohol, then two similar quantities of ether, each for one hour. All alcohol and ether extracts were decanted into a weighed 50 cc. flask, then evaporated, dried, and the lipides weighed. The remaining residue of non-extractable solids was dried overnight and weighed.

All chloride and potassium analyses were done by previously described methods (5). Table 4 shows the data on the lipide-free solids, and the chloride and potassium contents of the cortex, medulla and total kidney. The latter was cal-

culated from the concentrations present in the cortex and medulla and the weights of each portion.

The solid content of the kidney as a whole increased during dehydration, while during diuresis there was a decrease in the solids of the total kidney and the cortical portion, but little change in the solid content of the medullary portion. These changes in solids are in general similar to those found in muscle tissue.

The chloride content of the total kidney and its cortex showed little change on dehydration, but there was a definite increase in the medulla. In the rats having a water diuresis the chlorides were low in both portions of the kidney, while those belonging to the saline diuresis group had increased chlorides. It is apparent that

TABLE 4

Kidney

Average values for the lipide-free solids (gram/kgm.), chloride (mEq. Cl/kgm. lipide-free tissue), and potassium (mEq. K/kgm. lipide-free tissue). Number of animals indicated in parentheses.

		SOLIDS	Cl	K
Normal group	Cortex	201.1 (6)	58.70 (6)	73.23 (3)
	Medulla	162.9 (6)	81.36 (6)	72.09 (3)
	Total kidney	191.9 (6)	65.24 (6)	72.31 (3)
Dehydrated group	Cortex	212.9 (6)	57.18 (6)	78.61 (3)
	Medulla	172.6 (6)	91.26 (6)	69.57 (3)
	Total kidney	203.5 (6)	64.98 (6)	76.63 (3)
Distilled water diuresis group	Cortex	185.8 (6)	47.52 (6)	62.90 (3)
	Medulla	160.6 (6)	60.21 (6)	60.54 (3)
	Total kidney	178.1 (6)	51.47 (6)	62.00 (3)
Isotonic saline diuresis group	Cortex	191.1 (6)	65.20 (6)	63.02 (2)
	Medulla	163.4 (6)	87.56 (6)	55.64 (3)
	Total kidney	183.2 (6)	71.67 (6)	61.96 (2)

water excess causes a much greater loss of chlorides from renal tissue than from muscle tissue.

The potassium content of the entire kidney showed an increase on dehydration and a decrease during both forms of diuresis.

Calculations. From these determinations of tissue constituents, it is possible to calculate the partition of the tissue into its extracellular and intracellular compartments and the concentration of various substances in these compartments, providing the assumption is valid that the tissue chlorides are extracellular and the potassium largely intracellular. Though this assumption holds for muscle, it is probably not applicable to kidney tissue without making certain additional corrections for the presence of urine in the tissue analyzed, as developed below.

The extracellular fluid weight, E , was calculated as described by Hastings and

Eichelberger (8), that is:

$$E = \frac{\text{m.eq. Cl/kgm. tissue}}{\text{m.eq. Cl/kgm. serum water}} \times \frac{95}{99} \times 1000 \quad (1)$$

The total extracellular weight, E_T , was considered to be equal to the weight of the extracellular fluid plus collagen and elastin, which were assumed to be 8 gram/kgm. in muscle and 5 gram/kgm. in the kidney (9, 10). The intracellular weight per kilo of tissue, C , is then equal to $1000 - E_T$.

RESULTS. Derived Data—Muscle. In table 5, the values of the extracellular phase, E , of the intracellular phase, C , and of the intracellular concentration of water, $(H_2O)_C$, of the muscle tissues have been calculated according to the method just outlined. The data indicate clearly a decrease in extracellular fluid of 18 per cent after dehydration, an increase in extracellular fluid of 10 per cent after water excess, and an increase in intracellular fluid of 16 per cent after isotonic saline excess. There is, furthermore, a decrease in intracellular water in

TABLE 5

Muscle—Derived data

Average values of E and C (grams per kilo of lipide-free tissue) and $(H_2O)_C$ (grams per kilo of C). Number of animals indicated in parentheses.

	E	C	$(H_2O)_C$
Normal group	135 (4)	865 (4)	740 (4)
Dehydrated group	111 (5)	889 (5)	732 (5)
Distilled water diuresis group	149 (5)	851 (5)	763 (5)
Isotonic saline diuresis group	156 (4)	844 (4)	744 (4)

dehydration, an increase in intracellular water in water excess, and a negligible change after isotonic saline.

The three per cent shrinking of the cells in dehydration and the nine per cent swelling of the cells after water excess, amounts too small to be detected by microscopical examination, illustrate the value of quantitative histochemical studies of tissues in such experiments.

Derived Data—Kidney. The derived data for E , C , and $(H_2O)_C$ of the kidney calculated according to the method of Hastings and Eichelberger (7) are given in table 6. These data indicate a greater extracellular phase, E , in the medulla than in the cortex in all experiments. They also indicate that the extracellular phase of the cortex did not materially change as the result of dehydration or diuresis. It would appear from the data, however, that dehydration had resulted in a rather large increase in the extracellular phase of the medullary portion. Such a result is not consistent with what one might have expected, therefore the premises upon which these calculations were based were re-examined.

The basic assumption used in the above calculations, but which cannot justifiably be applied to the kidney, is that all of the chloride found in the tissue is

extracellular and is present in the extracellular fluid in a concentration equal to that of an ultrafiltrate of blood serum.

It is obvious that part of the chloride found in analysis of the kidney tissue was present in the urine contaminating the tissue. An attempt has, therefore, been made to evaluate the amount of urine in the tissue and from this, obtain a corrected value for the extracellular and intracellular phases of the cortex and medulla. These revised calculations were carried out as follows.

It was assumed that all of the chloride of the kidney is present either in the urine or the extracellular fluid, and that its concentrations in these two fluids equal those found by analysis of urine and serum.

TABLE 6
Kidney—Derived data

Average values of E and C (grams per kilo of lipide-free tissue), and $(H_2O)_C$ (grams per kilo C).

		E	C	$(H_2O)_C$
Normal group	Cortex	513	487	603
	Medulla	654	346	555
	Total kidney	530	470	601
Dehydrated group	Cortex	509	491	581
	Medulla	755	245	299
	Total kidney	559	441	557
Distilled water diuresis group	Cortex	496	504	646
	Medulla	629	371	594
	Total kidney	537	463	632
Isotonic saline diuresis group	Cortex	528	472	611
	Medulla	704	296	481
	Total kidney	580	420	583

One may then set up a series of three equations from which the values of the extracellular phase, E , the intracellular phase, C , and the urine, U , may be calculated. These equations are:

$$E + C + U = 1000 \quad (2)$$

$$1000 \text{ Cl}_T = (\text{Cl})_U \times U + (\text{Cl})_E \times E \quad (3)$$

$$1000 \text{ K}_T = (\text{K})_U \times U + (\text{K})_C \times C + (\text{K})_E \times E \quad (4)$$

The values of K_T and Cl_T are found by analysis of the kidney tissue, $(\text{Cl})_U$ and $(\text{K})_U$ by analysis of the urine, and $(\text{Cl})_E$ by analysis of the serum. The values of $(\text{K})_C$ and $(\text{K})_E$ are assumed to be constant and to have the values of 120 mEq. per kilo of cells and 4 mEq. per liter of extracellular fluid, respectively. The equations are then solved for E , C and U . These revised values are given in table 7.

From these revised figures we see that the cellular phase, C , constitutes about 45 per cent of the cortical portion of the kidney and is increased during both diuresis and dehydration, but to a greater degree in the latter. In the medulla, the cellular phase constitutes about seventeen per cent of the normal kidney, twenty-seven per cent of the dehydrated kidney, and about forty-six per cent of the kidney which is undergoing diuresis. It is possible that the larger cellular phase in the medulla of kidneys which are excreting excess fluid is due to the swelling of these medullary cells as the larger amount of hypotonic fluid passes into them from the tubules. These calculations lead to unreasonably low, and even negative values for the urinary phase in the case of distilled water diuresis. The presence of intracellular chloride would account for such negative results.

TABLE 7
Kidney
Revised values of C , E and U (gram/kgm.)

		C	E	U
Normal group	Cortex	448	348	204
	Medulla	166	257	577
	Total kidney	369	329	302
Dehydrated group	Cortex	535	352	113
	Medulla	274	421	304
	Total kidney	475	367	158
Distilled water diuresis group	Cortex	507	486	6
	Medulla	487	655	-143
	Total kidney	500	539	-39
Isotonic saline diuresis group	Cortex	507	400	93
	Medulla	436	129	435
	Total kidney	495	283	221

It is possible to calculate the concentration of intracellular water by the following method. The total lipid-free kidney solids, S_T , are equal to the sum of the cell solids, S_C , the extracellular fluid solids, S_E , the collagen and elastin, S_{Co} , and the urinary solids, S_U . S_T is known by analysis; S_U is equal to the product of the urinary phase, U , and the concentration of solids in the urine $\div 1000$; S is equal to the product of the extracellular phase, E , and the concentration of its solids (10 gram/kgm.) $\div 1000$ and S_{Co} is equal to 5 gram/kgm. tissue (by assumption). The cell solids, S_C , are then readily calculated. The intracellular water, H_2O_c , is equal to the difference between the cellular phase, C , and the cell solids; the concentration of intracellular water per kilo of cells, $(H_2O)_c$, is $H_2O_c/C \times 1000$. These values are shown in table 8.

According to these calculations the intracellular water concentration of the kidney increases during both dehydration and diuresis. This change is more pronounced in the medullary portions than in the cortical portions. This would

appear to be additional confirmatory evidence that the medullary cells are swollen by the passage of water into them as it is salvaged from the urinary phase for the maintenance of the body fluid.

When we compare these derived values for the cellular water concentration with those in table 6 calculated by the method of Hastings and Eichelberger (8), we find that the new values are in general somewhat higher, the majority being around 65 per cent. Manery and Hastings (1) came to the conclusion that reasonable values for the intracellular water were between 70 and 75 per cent. Therefore, it seems apparent that the present method of calculation leads to values of $(H_2O)_c$ which are more consistent with the present knowledge of the histochemical composition of tissues. It does not, however, preclude the possibility of some intracellular chloride in kidney tissue.

DISCUSSION. The physiological and histological complexity of renal tissue introduces many difficulties which are not encountered in the analysis of other more simple and more homogeneous structures. The urine electrolytes as measured in the collected specimen are probably not a true representation of the varying electrolyte concentrations which exist in the renal tubules. In addition, the

TABLE 8
Kidney—Derived data
Intracellular water concentration, $(H_2O)_c$, (gram/kgm. C)

	CORTEX	MEDULLA	TOTAL KIDNEY
Normal group.....	616	433	590
Dehydrated group.....	652	582	643
Distilled water diuresis group.....	654	679	661
Isotonic saline diuresis group.....	654	709	677

assumption that chloride is mainly extracellular and potassium intracellular is probably not valid for the kidney as these substances at times must pass through the tubular cells and reenter the extracellular fluid. The fact that the electrolyte relationships of the medullary portion differ from those of other tissues, such as muscle, is supported by the figures which have been presented. The cortical portion, however, would appear to have an electrolyte pattern more closely approaching that of other tissues and is evidently less influenced by the physiological activity of the kidney.

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SUMMARY

1. An attempt has been made to analyze the histochemical relationships of the kidney and the effect of dehydration and diuresis on these relationships. The accompanying changes which occur in the muscle, the blood and the urine are also presented.

2. A new method of calculation of these histochemical relationships has been

proposed which includes the urinary phase in addition to the intracellular and extracellular phases. More reasonable values for the intracellular water are obtained by this method.

3. Histochemical analysis is offered as a new means of approach to the investigation of the complexities of renal physiology.

REFERENCES

- (1) MANERY, J. F. AND A. B. HASTINGS. *J. Biol. Chem.* **127**: 657, 1939.
- (2) EICHELBERGER, L. AND W. G. BIBLER. *J. Biol. Chem.* **132**: 645, 1940.
- (3) LINDERSTROM-LANG, K. AND H. LANZ, JR. *Compt.-rend. trav. Lab. Carlsberg, Serie chim.* **21**: 315, 1938.
- (4) JACOBSEN, C. F. AND K. LINDERSTROM-LANG. *Acta Physiol. Scand.* **2**: 149, 1940.
- (5) LOWRY, O. H. AND A. B. HASTINGS. *J. Biol. Chem.* **143**: 257, 1942.
- (6) BODANSKY, M. *Introduction to physiological chemistry*. 4th ed., p. 449. John Wiley and Sons, N. Y., 1938.
- (7) MOORE, N. S. AND D. D. VAN SLYKE. *J. Clin. Investigation* **8**: 337, 1930.
- (8) HASTINGS, A. B. AND L. EICHELBERGER. *J. Biol. Chem.* **117**: 73, 1937.
- (9) LOWRY, O. H., A. B. HASTINGS, T. Z. HULL AND A. N. BROWN. *J. Biol. Chem.* **143**: 271, 1942.
- (10) LOWRY, O. H. Unpublished data.

MECHANISMS UNDERLYING ELECTROCARDIOGRAPHIC CHANGES OBSERVED IN ANOXIA

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Of the electrocardiographic changes which develop in man and experimental animals subjected to low oxygen tensions the changes in T and S-T have received the most attention. In the anoxic dog (8) these changes differ in direction from those in the human (11), but they are equally striking. In both the dog and the human the changes have been attributed to anoxia of the myocardium.

Another change, a reduction of the height of the R wave in all three conventional limb leads, has been observed in human electrocardiograms beginning at various altitudes between 5000 and 20,000 feet (1), but at these levels changes in T and S-T were found to be small and inconstant. Seeking to find an early sign of change associated with oxygen want, Randall (10) recently studied the changes in the ECG of the dog as the O₂ content of the inspired air was slowly reduced by rebreathing. He recorded a reduction in the height of R in all limb leads, usually accompanied by flattening and splintering. The changes were greatest in lead III. The possibility that these changes were early signs of impairment in conduction was considered, but this interpretation was rendered improbable by the associated findings of slightly reduced P-R intervals and reduced duration of QRS. These intervals were lengthened only at the crisis. Changes were noted in the T and ST records also, confirming observations of others. ECG's alone are insufficient to explain any of the electrocardiographic changes that occur in dog or man.

The present experiments were designed to attack these problems directly, correlating with the ECG other data from local leads on the hearts of open chested preparations and measurements of changes in the size of the thorax in closed chested animals under the influence of anoxia and in other conditions that appeared to simulate the changes seen in anoxia.

Animal preparations and recording. Sixteen dogs were used. They were anesthetized with morphine sulfate (about 2 mgm. per kilo) and Na barbital (180 mgm. per kilo). In the experiments using direct leads, the chests were opened by midline incision of the sternum. Usually the heart was suspended by sewing the opened pericardium to the retracted edges of the chest opening.

For simultaneous multiple electrographic registration, three string galvanometers and, in some experiments, a G. E. Victor Electrocardiograph were arranged to record on the same camera. The speed of the photographic paper in local

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recording experiments was 150 mm. per second, or 6 times the usual rate for electrocardiography, and in other experiments, 90 mm. per second. Bipolar contiguous electrodes whose construction and properties have been described (6) were used for direct local leading. For electrocardiograms, metal plates covered with cotton saturated with Locke's solution were implanted under the skin of the left forelimb and the left thigh. Since it has recently been shown that the changes in anoxia are qualitatively alike in all leads, but greatest in L-III, these experiments have been concentrated upon L-III.

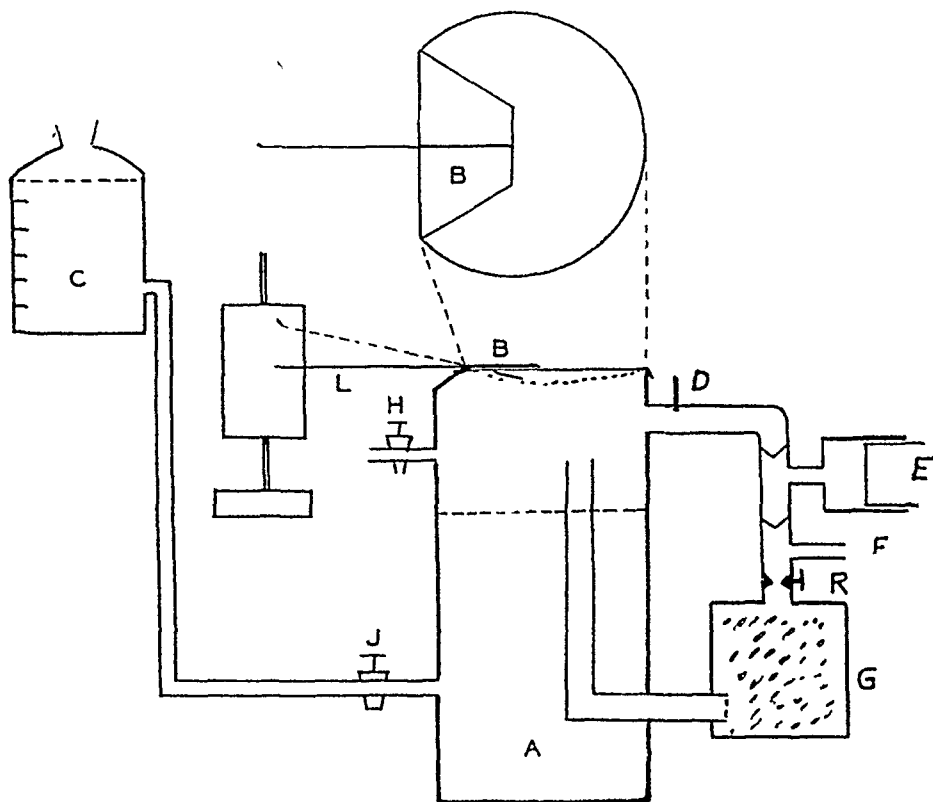


Fig. 1. Burlage and Wiggers' rebreathing respirometer with the additions that were required for its use with artificial respiration. See text.

Natural respiratory movements and artificial inflation and deflation were registered along with the electrograms in many of the experiments by means of a shadow casting lever attached to the membrane of a tambour. The tambour was activated by the pressure changes in either a pneumograph or pleural cannula during the parts of experiments when the animals' chests were closed, and by a side tube from the artificial respiration line during open chest procedures.

Respiratory apparatus. The device used for the control of the oxygen content of inspired air during rebreathing procedures was a modification of the respirometer of Burlage and Wiggers which was described by Randall (10). The modifications required for open chest experiments were those incident to the addition of artificial respiration to this closed circuit system (see fig. 1). The principal change was the addition to the circuit of a piston pump *E* with an adjustable

stroke volume, the variation being made by changing the length of the stroke by means of an adjustable lever. It was found necessary to include an adjustable resistance R in the line distal to the connection to the animal's trachea, F . Without this, the animal's chest would not be inflated. The air would pass freely through the small resistance of the soda lime, G , leaving the animal with grossly insufficient respirations. Oxygen content is expressed in percentage units. All experiments were done at laboratory atmospheric pressures, i.e., about 750 mm. Hg.

During the reduction of O_2 by rebreathing, water was added as O_2 was removed, thus keeping the record on the long paper kymograph L working on the same horizontal line throughout. The per cent of O_2 could be read approximately from the water bottle C at any moment. Samples were taken periodically for confirming analyses. The gas analysis results are the figures reported. After the O_2 content of the respirometer tank had been reduced down to the desired minimal percentage by rebreathing, it was stabilized at that percentage by adding oxygen from the tank attached at H , the oxygen being admitted at a rate just equal to that of its utilization.

RESULTS. *Interpunctal and electrocardiographic intervals, and changes in voltage in local spikes and in R of the ECG, recorded from animals with open chests.* From simultaneously made records of two or three direct leads and an ECG measurements were made of the intervals between spikes from direct leads on two well separated placements; of the durations of the P-R and QRS portions of the ECG; and of the heights of the spikes from local leads, and of R of the ECG. In any reduction of conduction rate within the ventricular system the interpunctal intervals between local leads might be expected to increase and the width of QRS should increase. A slowing of A-V conduction would increase the P-R interval. If the reduction in the height of the R complex, observed in moderate anoxia, represents real reductions in the voltage of action potentials of the muscle then the spikes in the records from direct leads should be correspondingly reduced. To test for the occurrence of such changes, a record was made approximately at the end of each 15 minutes during the time that the oxygen content of respired air was being reduced by rebreathing and while the chosen low oxygen mixture was maintained. The results of the measurements in the different experiments are fairly represented by the data from two experiments shown in table 1.

In experiment 3 the interpunctal intervals were measured between leads on the early part of the right ventricle near the septum and the anterior left ventricle left of the apex. In experiment 9 local leads were on the right ventricle near the septum and on the left ventricle midway between the anterior descending artery and the whorl. In experiment 3 a diminution of the interval between onsets of local spikes by 1.5 msec. is shown at about 10 per cent O_2 and a lengthening occurs when the O_2 is between 8 and 6 per cent. During this period of reduced interval (10 to 8 per cent and slightly beyond), a shortening of the P-R and QRS intervals of the ECG is shown also. In experiment 9, the diminutions appear in the 12 per cent line, but a return essentially to the control figures is seen at $7\frac{1}{2}$ per cent. The diminutions are not great, but they occur, and they may be interpreted as

indicating that there is no retardation in conduction during anoxia down to 8 per cent or slightly less. On the contrary, the process appears to be slightly accelerated. The voltage of local spikes remains essentially constant at all O₂ concentrations down to less than 8 per cent. In experiment 3 there was a decline in one local lead 30 minutes after the measurement at 8 per cent at a time when analysis showed 6.5 per cent O₂, and in the other lead only after 15 additional minutes in the 6.5 to 7 per cent range. In experiment 9 the first reductions occurred after about 10 minutes at the 7.5 per cent stage. The voltage of R in the ECG of the open chested animal is shown to be equally resistant to reduc-

TABLE 1

Changes in interpunctal and electrocardiographic intervals, voltages and arterial pressure associated with reduced O₂ in inspired air

TIME HRS.:MIN.	%O ₂	B P.	DURATIONS, MSEC.			SPIKE HEIGHT, MM.		
			Local intervals	P-R	QRS	R. Pt.	L. Pt.	R-ECG
Expt. 3								
0	20.9	115	17.0	77	47	14	12	5
0:30	15	105	17.0	77	47	13	12	5
1:00	10	120	15.5	74	44	14	13	5
1:25	8	120	15.5	71	44	14	12	5
1:40	6.5	90	16.5	71	42	14.5	12	5
1:55	6.5	75	18.0	74	44	14.5	9	5
2:10	7	70	19.5	78	45	8	10	5
2:30	7	40	19.5	91	52	8	9	4
2:32	7	20	21	108	85	8	9	4
Block and failure								
Expt. 9								
0	21.9	95	12	56	33	19	12.5	10.5
20	16	95	12	56	33	19	12.5	10.5
35	12	110	11	51	31	19	13	10.5
55	7.5	40	12	54	35	19	13	10.5
1:05	7.5		17	97	74	10	11	2?

The time measurements were made with the aid of a magnifier and a 3 msec. scale. Errors of reading are within limits of ± 1 msec.

tion by anoxia. No reductions are seen above the 7 and 7.5 per cent measurements. This is in sharp contrast to the changes in the height of R from animals with unopened chests which show a marked reduction in the region of 11 to 8 per cent O₂ as previously reported (10) and confirmed in experiments to follow.

Upon direct observation of hearts in the 10 to 8 per cent O₂ zone they are seen to be very cyanotic and usually appear small and contracting vigorously. When cardiac failure occurs a considerable and sometimes rather sudden dilatation is seen. This is followed quickly by incomplete and then complete heart block and standstill, as described by Greene and Gilbert (3). More gradual dilatation is seen in some cases.

The tolerance of the heart and circulatory system to anoxia shown in experi-

ment 3 is more nearly typical of the majority of dogs studied than is that in experiment 9. The difference was manifest chiefly in the duration of the low oxygen period endured before circulatory and cardiac failure supervened. In no. 3 the oxygen content of inspired air was held at the 6.5 to 7 per cent level for a period of approximately 75 minutes before heart block and failure occurred, while in no. 9 a comparable O_2 level produced block and failure within a period of 15 minutes or less. One animal showed still less tolerance, block and failure occurring at 9.4 per cent O_2 . This level had been maintained for 12 to 15 minutes. This animal probably belongs in the "nontolerant" group (10). The reason for the differences is not known. Of the ten dogs with artificial respiration from which data are available one failed at 5.0 per cent O_2 , one at 5.6, two at 6.0, three at 7, one at 8 and one at 9.4. The usual barbitalized dog with artificial respiration can maintain life at an 8 per cent O_2 level for an hour or longer. Below 6 per cent the period usually is limited to very few minutes.

The blood pressure changes during progressive anoxia were very much alike in one feature. In every experiment except one there was a rise above the control pressure when the O_2 content was between 12 and 9 per cent. As O_2 was further diminished, the pressure progressively fell from this peak. During the early mild anoxic period of 18 to 13 per cent in some experiments there was an initial decline in pressure followed by the rise at 12 to 9 per cent and the subsequent fall.

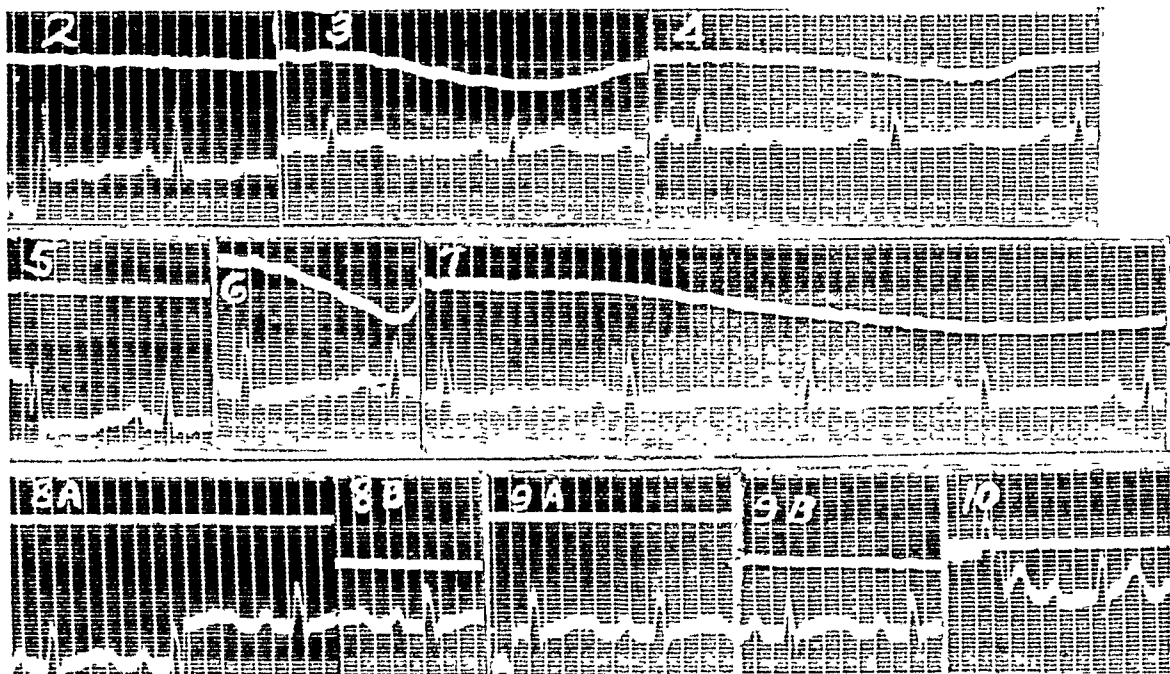
The beginning of the final decline in arterial pressure definitely preceded any obvious cardiac dilatation, the lengthening of any intervals beyond the normals or the decline of potentials.

The foregoing observations by direct leading revealed no changes in conduction or voltage which could explain the flattening and splintering of the R wave in moderately anoxic dogs with closed chests, nor have flattening and splintering of R complexes been observed in anoxic dogs with open chests. Therefore, experiments were devised to seek the cause outside of the heart. Since hyperpnea to at least two or three times the normal minute volume commonly occurs in breathing an atmosphere with considerably reduced oxygen content it was logical to look to hyperpnea and to physical changes which accompany it for possible causes. For these experiments dogs with closed chests and spontaneous respiration were used.

Effects of anoxic and CO_2 hyperpnea and artificial inflation on the ECG. Figures 2, 3 and 4 show the lead III electrocardiograms and respiratory tracings of a dog breathing a normal atmosphere in figure 2, after rebreathing down to 8.2 per cent O_2 in figure 3 and after returning to air for 5 minutes in figure 4. In the control period, the height of R is 11 mm. in inspiration and 12 mm. upon expiration. In figure 3 the height of R is 4 mm. during inspiration and 6 mm. during expiration. In figure 3, also, the steepness of the rise of R shows some reduction and the summit is flattened. The S-T interval and T show an increased elevation. In figure 4 there is partial recovery of the control height and shape of R, while T and S-T have lost some of the elevation that they gained during anoxia. Figure 5, made after 23 minutes on air, shows apparently complete recovery.

At the 8.2 per cent stage the respiratory rate was about three times the control, and the depth, though irregular, averaged near that of the control. This type of shallow rapid respiration is seen frequently in anoxia and has been described as typical of anoxic hyperpnea in the human (5), although there are exceptions.

The maintenance of a small tidal volume could obviously be correlated with the smallness of the variation in the height of R associated with the phases of respira-



Figs. 2 through 10. Respiratory record above and lead III electrocardiogram below. Inspiration or inflation downward. Reduced to $\frac{1}{2}$ original size.

Fig. 2. Control ECG with quiet respiration of normal atmosphere.

Fig. 3. After rebreathing down to 8.2 per cent O_2 , CO_2 absorbed.

Fig. 4. Five minutes after return to air.

Fig. 5. Twenty-three minutes after return to air.

Fig. 6. During CO_2 hyperpnea.

Fig. 7. During artificial inflation without anoxia.

Fig. 8. Another experiment. Control on normal atmosphere; A, during expiration; B, inspiration.

Fig. 9. Same experiment after rebreathing down to 8.5 per cent O_2 ; A, expiration; B, inspiration.

Fig. 10. After rebreathing to 5.8 per cent. Respiration had stopped.

tion. But the overall change in height and shape is far greater than the phasic variation. To explain this on a basis of inflation would require a maintained inflation, i.e., an increased expiratory volume of the chest. Gesell and Moyer (2) have reported an increased expiratory circumference of the chest in anoxic dogs, and an increase in the expiratory volume of the human chest during air hunger and dyspnea has been reported by Greene and Swanson (4). The expiratory girth of the chest was measured at frequent intervals during the development of anoxia in our later experiments. During anoxia the expiratory

girth of the dog's chest unfailingly increases. The usual amount of this increase in an 8 kilo dog at 10 to 8 per cent O_2 is 10 to 15 mm. During normal quiet respiration the phasic changes in girth from inspiration to expiration (tidal changes) are from 1.5 to 3 mm., therefore a 15 mm. change in expiratory circumference is a large change. During hyperpnea from anoxia the tidal changes seldom are greater than 5 mm., but during the very deep respirations of CO_2 hyperpnea tidal changes as great as 25 mm. have been measured. The expiratory girth of the animal in CO_2 hyperpnea is no larger than that of the control measurement and may be 3 or 4 mm. smaller, thus differing markedly from the expiratory size in anoxia.

Corresponding with the failure of the expiratory circumference to increase in CO_2 hyperpnea, figure 6 shows that the ECG remains normal during the expiratory phase, while suffering a diminution and flattening of R during the inspiratory phase. Figure 7 is a record of artificial inflation of the dog with closed chest by

TABLE 2
Relation of thoracic girth to height of R_{III}
(From experiment illustrating fig. 2-7)

	GIRTH	HEIGHT OF R
	cm.	mm.
Anoxia Control, Expiration.....	39.7	12.5
8.2 per cent O_2 , Exp.....	41.6	6.5
Insp.....	41.9	5.5
CO_2 hyperp. exp.....	39.7	12.0
Insp.....	42.2	8.0
Art. Resp. deflation.....	40.0	11.5
Inflation.....	41.5	6.5

use of the artificial respiration apparatus. Here also, it is seen that during inflation R diminishes markedly in height and is deformed in a manner resembling that of anoxia in figure 3. S-T and T remain unchanged during CO_2 hyperpnea and artificial inflation.

Quantitatively, the reduction in height of R with a given overall increase in chest girth is greater in anoxia than during the deep inspiration of CO_2 hyperpnea, but equal to that of artificial inflation. The lowering and distortion of R are greatest when the chest is largest. After returning the anoxic animal to air the change in R and the increased girth decrease and disappear together. In an occasional animal there is little change in R with anoxia. In these there is little change in expiratory girth. Figures 8, 9 and 10 are from such an experiment. The measurements of girth and the simultaneous heights of R in the experiment from which figures 2-7 were taken are shown in table 2. The close correspondence between girth and height of R in the slower changes in inflation is very striking. The divergence from this relationship in the quick violent inspirations

of CO₂ hyperpnea probably means that in these fast movements there is insufficient time for physical equilibria between intrathoracic pressures and positions of organs to be achieved.

It was pointed out earlier that S-T and T in figure 3 (8.2 per cent O₂) were elevated above the control record, figure 2. Figures 8, 9 and 10 from another experiment show this elevation with anoxia more emphatically. Figures 8a and 8b are control records of expiration and inspiration respectively, while breathing air. Figures 9a and 9b are expiration and inspiration after rebreathing O₂ down to 8.5 per cent, and 10 was made just after respiratory movements stopped. The O₂ content of the tank was 5.8 per cent. In this experiment there is no elevation of S-T at 8.5 per cent, but at 5.8 per cent there is a large increase in the slope of S-T and in the height of T which rose from 2 mm. to 5.5 mm.

The elevation and widening of T with greater steepness of slope of S-T occurs in all or almost all cases of anoxia in dogs with closed chests if T was upward in the control. In the experiments with an inverted T, the inversion disappeared and in some cases was replaced by a small positive wave.

In the open chested animals, changes in T with anoxia were never large and striking as in closed chested preparations, but they occurred. The direction of the changes, like those in the unopened chests, were upward, and in case of inversion in the control the inversion usually disappeared, though in one case it remained and the downward T merely widened. In both open and unopened preparations there were wide variations in the lowering of the O₂ percentage required to produce the first detectable change in T. Changes came as early as 10.5 per cent in some cases, but usually were not marked until the per cent of O₂ reached a level below 9.

DISCUSSION. The reduction in the height of R, especially R_{III} in anoxic dogs, together with the flattening and splintering that commonly accompany the reduction have been shown to be associated with the enlargement of the thorax. Since the changes are roughly proportional to changes in chest girth and can be duplicated by artificial inflation when the animal is fully oxygenated, these changes can be regarded as extrinsic to the heart itself and therefore without value as an indicator of the tolerance of the heart to anoxia. The reduction and distortion are great at O₂ levels well above the range of 7 per cent and below at which intrinsic reductions do occur. At these lower levels rapid dilatation and failure are always imminent. Real reductions in voltage shown by direct leads may be regarded as terminal events.

The fundamental reason for the changes in R in the dog with expanded chest and with or without moderate anoxia could be conceived as resulting from changed conductivity between the heart and the body wall, or as resulting from a changed position of the heart which would rotate its electrical vectors. A number of observations combine to rule out a simple resistance or conductivity change as playing any major rôle. A change due to increased resistance should be present and in the same proportion in all deflections of the ECG. The record shows that when R is reduced markedly by inflation or anoxia, P is not cor-

respondingly reduced. The same may be said for T, though T deserves special mention which is given later. The change in R itself is not a symmetrical reduction, but a distortion.

Rotation of the heart with its consequent change in the relation of the QRS vectors to the plane of the leads is the probable answer. Randall's records have abundantly shown that the reduction of R occurs in all three leads. Therefore, there are no reciprocal changes in leads I and III, and the alterations are not explainable as right or left axis deviation. In the human, the records of Katz et al. (7) and of others have shown that the diminution in the height of R in all leads occurs in some anoxic individuals, but not in all. In those with reduced R in all leads, the QRS changes resemble those of the dog with expanded chest. Some of their other human subjects showed QRS changes in the direction of right axis deviation or the reduction of left axis deviation (compare Katz' figs. 1 and 3 with 5 and 6). This latter is the change most frequently seen in deep human inspiration. In the light of the ECG findings in dog and man and the previously referred to reports (4) that the human, like the dog, exhibits chest expansion upon the development of anoxia, we may summarize by saying that upon enlargement of the thoraces of dogs and some men their hearts in all probability undergo a displacement of a kind which reduces R in all leads and produces some distortions, the greatest changes being in lead III. This appears to adequately account for the change in R of the ECG of the dog and perhaps the human with similar changes at O_2 levels down to about 7 per cent.

The changes in T and S-T do not parallel those of R. In the dog as anoxia develops T becomes higher, whereas R becomes lower and these changes fail to correspond in time of onset and in quantity. S-T and T are not changed by artificial inflation or deep inspiration, but R is reduced and deformed by them. Therefore, the changes in R and those in T and S-T must have different causes. The fact that in open chested animals anoxia produces some elevation and widening of T, while there was no change in R, supports this view and seems to indicate that moderate anoxia of the heart does produce a change in T and S-T as reported by Kountz and Gruber (8).

May (9) found that the decline in T in the human ECG with anoxia was greatest in young athletic individuals and that it was very small in the aged. They inferred that the T change is associated with the adaptation of the heart to greater stress. In some of our animals the changes did not occur until levels ordinarily associated with obvious dilatation and the beginning of failure were reached, but in others they began at 10 to 11 per cent when the blood pressure was high. A possible interpretation is that the T wave changes are signs of greater cardiac stretch whether it be within normal limits and compensated by contractions of greater amplitude or uncompensated and associated with a weakened myocardium. Since the anoxic changes in T in dog and man are in opposite directions one might question the supposition that they have similar causes. But the changes become noticeable in the published human records at about the same O_2 levels as in some of our records from dogs.

We may conclude that the height of the R complex is no indicator of cardiac

response to anoxia. It is a result of asymmetrical respiratory stimulation (increased inspiratory tonus). The change in T, though apparently of cardiac origin, cannot at present be interpreted as a sign of cardiac damage or danger in anoxia, though further quantitative studies might give it value as a sign.

SUMMARY

Direct leads from the ventricles of dogs with open chests and simultaneously recorded electrocardiograms show no lengthening of interpunctal intervals or of P-R or of the duration of QRS at any level of anoxia to below 8 per cent in inspired air. On the contrary, there is often a small diminution of intervals during moderate anoxia. At 7 per cent O_2 and below, with cardiac dilatation and failure, the intervals do lengthen.

There is no reduction in voltage of local leads or of R of the ECG at any O_2 level down to below 8 per cent.

Dogs with artificial respiration vary considerably in their tolerance to anoxia. In the usual case, a level of 8 per cent can be tolerated for an hour and longer. However, one animal out of ten so treated failed at 9.4 per cent after about 15 minutes at this level.

With the development of anoxia, the animal with closed chest and spontaneous respiration shows reduction in height of the R complex and usually a distortion, flattening of the summit, or splintering. These changes occur at the comparatively safe levels of 11 to 8 per cent O_2 .

As anoxia develops the girth of the chest during expiration is increased. The reduction in the height of R is roughly proportional to the increase in girth. The change in R can be produced without anoxia by artificially inflating the chest. It is not due to changed resistance, but very likely to rotation of the heart.

The elevation in T which occurs in anoxia cannot be produced by inflation and it appears to be of cardiac origin. It is probably due to a dilatation of the heart, compensated or uncompensated.

Certain correlations between observations on animal and human electrocardiographic changes in anoxia are attempted.

REFERENCES

- (1) BENSON, O. O. *J. Aviation Med.* 11: 67, 1940.
- (2) GESELL, R. AND C. MOYER. *Quart. J. Exper. Physiol.* 24: 332, 1935.
- (3) GREENE, C. W. AND N. C. GILBERT. *This Journal* 60: 155, 1922.
- (4) GREENE, J. A. AND L. W. SWANSON. *Arch. Int. Med.* 61: 720, 1938.
- (5) HALDANE, J. S. *Respiration*, p. 118, 1922.
- (6) HARRIS, A. S. *This Journal* 134: 319, 1941.
- (7) KATZ, L. N., W. W. HAMBURGER AND W. J. SCHUTZ. *Am. Heart J.* 9: 771, 1934.
- (8) KOUNTZ, W. B. AND C. M. GRUBER. *Proc. Soc. Exper. Biol. and Med.* 27: 170, 1929.
- (9) MAY, S. H. *Am. Heart J.* 17: 655, 1939.
- (10) RANDALL, W. C. *Am. Heart J.* 27: 234, 1944.
- (11) WHITE, M. S. *J. Aviation Med.* 11: 166, 1940.

OBSERVATIONS ON METHODS OF INCREASING RESISTANCE TO OXYGEN POISONING AND STUDIES OF ACCOMPANYING PHYSIOLOGICAL EFFECTS¹

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The first report of oxygen poisoning was that of Paul Bert who described convulsions in animals exposed to oxygen under increased pressures (1). The primary effect of continuous inhalation of pure oxygen at sea level atmospheric pressure is the production of congestion and edema in the lungs, which was described by Lorraine Smith (2) and subsequently by Hill (3), Karsner (4) and others. In a recent extensive review of oxygen poisoning Stadie, Riggs and Haugaard (5) concluded that respiratory symptoms predominate as a result of the action of oxygen at 0.8 to 2 atmospheres, while central nervous symptoms occur at higher pressures. It has, however, been repeatedly noted that a lethargic state is observed in dogs, rabbits and other animals during the first twenty-four hours' inhalation of 100 per cent oxygen (Binger, Faulkner and Moore (6)).

Barach (7) was not able to increase resistance to oxygen poisoning in rabbits by gradual increase in the concentration of oxygen in the inspired air. Drinker and his co-workers (8, 9) carried out studies on rats exposed to four atmospheres of air pressure in which progressive cellular hypertrophy and hyperplasia of the alveolar walls were found as a result of the increased oxygen pressure. These changes persisted for months after return to normal pressure, and occurred in both young and old rats, but to a greater extent in the older ones. The histologic structure resembled an exaggerated form of that normally found in unexposed young rats. The animals that had been previously exposed, as well as the younger unexposed animals, revealed an increased tolerance to high air pressures equivalent to 0.8 atmosphere of pure oxygen. The possible protective function of these structural changes was additionally suggested by the presence of thickening of the walls of the pulmonary arterioles. Hederer and Andre (10) reported a greater tolerance to oxygen in young than in older rabbits but found an increased sensitivity rather than increased tolerance with previous exposure; the latter finding was reported by Almeida (11). Sayers (12) reported that rabbits could live for at least six weeks in 100 per cent oxygen when exposure was limited to sixteen out of the twenty-four hours. Barach and Soroka (13) confirmed this but noted that chronic changes were present in the lungs of these animals, characterized by thickening of the alveolar wall, infiltration of the alveoli with mono-

¹ The work described in this paper was done under a contract (OEMemr-47), recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the College of Physicians and Surgeons, Columbia University, New York City.

nuclear cells and evidence of scattered organizing pneumonia.² Paine, Lynn and Keys (14) showed that exposure to air part of the time increased the length of life of dogs in 100 per cent oxygen.

Increased concentration of carbon dioxide increases the toxicity of oxygen at several atmospheres of pressure. Gesell (15) states that oxygen at high tensions enhances the sensitivity of the animal to the administration of carbon dioxide, owing to the broken co-ordination of the dual function of hemoglobin, which leads to an acidosis. Behnke and others (16) showed that subnormal levels of alveolar carbon dioxide tension, produced by artificial hyperventilation, decreased the severity of oxygen poisoning, whereas increased percentages of carbon dioxide in the mixture hastened the convulsive effect of oxygen at elevated atmospheric pressures. Campbell (17, 18) reported that inhalation of increased concentrations of oxygen resulted in an increase in tissue oxygen pressure and carbon dioxide pressure.

In the human subject, Evans (19) and Boothby, Mayo and Lovelace (20) have reported that inhalation of pure oxygen by mask for periods of two to four days takes place without apparent pulmonary irritation. However, this does not necessarily indicate that the adult human being is not sensitive to oxygen poisoning since the mask is from time to time removed for feeding and other reasons. Becker-Freysang and Clamann (21) reported nausea and malaise at the end of sixty hours in 90 per cent oxygen, and on the third day the development of broncho-pneumonia in one of the experimenters. Charles C. Chapple (quoted by Stadie et al. (5)) found a high tolerance to 85 per cent oxygen in premature infants. He treated premature infants weighing less than three pounds for periods as long as three weeks in this high oxygen concentration, maintaining a relative humidity close to 100 per cent, without observing any signs of oxygen poisoning.

Barach and Richards (22) found that patients with pre-existing *severe chronic* anoxia after twenty-four to forty-eight hours' residence in an atmosphere of 50 per cent oxygen may develop a subdued and at times comatose state with headache and irrationality. In cases of chronic pulmonary disease, such as pulmonary fibrosis and emphysema, there is a progressive and marked increase in the carbon dioxide content of arterial blood, accompanied by an increased elimination of chloride ions and a decrease in pulmonary ventilation. Continuation of exposure to 50 per cent oxygen in these patients is accompanied by a disappearance of the irrational and comatose state, despite continued high levels of carbon dioxide. In patients whose respiratory function later improves, the carbon dioxide level in the blood becomes progressively lower. The pH of the blood in these cases is shifted very slightly to the acid side at the start of treatment but very soon maintains itself at or near the normal level (Richards and Barach (25)). The increased carbon dioxide tension in arterial blood makes possible a swifter elimination of carbon dioxide with a lowered volume of ventilation.

The idea that the action of pure oxygen is upon the enzyme system of cells is supported by many observers. Stadie et al. (5) summarize the data on enzy-

² Dr. Homer D. Kesten has assisted us in the interpretation of the pathological findings in this and the present study.

matic systems in relation to oxygen poisoning by stating that the toxic action of high pressures of oxygen will probably be explained in the light of inhibitory actions on enzymes with resultant severe disturbance of essential metabolic cellular reactions: "The evidence in the literature is scanty and non-systematized, but is sufficient to suggest the following possible modes of action of oxygen: 1, Oxidation of a co-enzyme to the inactive oxidized form; 2, oxidizing activating sulfhydryl compound; 3, oxidizing active —SH groups of enzyme molecule proper; 4, oxidation of metallo-hemochromogen to inactive oxidized form; 5, oxidizing activating metal constituent; 6, formation of inhibitor from precursor other than the enzyme; 7, inhibition of enzymatic activity by changing oxidation-reduction potentials of medium."

METHODS. In the case of small animals, such as rats, exposure to the oxygen atmospheres used was obtained by placing them in their usual cages inside a large closed chamber through which oxygen from a cylinder was passed at the desired rate, varying according to the number of animals employed. Residence in the oxygen chamber was usually continuous except when the chamber was briefly opened at intervals of about three days for provision of water and food. The time of exposure to air was not longer than fifteen minutes, following which the chamber was rapidly purged through a needle valve with 100 per cent oxygen. The carbon dioxide content of the chamber was kept below 0.5 per cent by a flow of oxygen of from 3 to 12 liters per minute (usually 3 liters of O_2 per rat per min.), with the addition of Baralyme spread on the floor of the chamber and shell natron exposed in shallow vessels. The atmosphere in the chamber was tested once a day for oxygen and carbon dioxide. Since some carbon dioxide was usually present, the actual concentration of oxygen in the chamber was approximately 99.1 to 99.3 per cent with about 0.4 to 0.5 per cent carbon dioxide.³ The chamber was kept at ordinary room temperatures by water circulating through coils in the chamber walls.

The method most commonly used to "acclimatize" the animals to 100 per cent oxygen was that of an increase by stages in the oxygen content of the atmosphere to which they were exposed. The rate of increase in oxygen concentration prior to reaching 100 per cent was varied somewhat in a number of preliminary experiments without significant change in the subsequent result. The stages in the "acclimatization" period were usually as follows, the oxygen concentration being varied by using the injector of the Meter oxygen mask: 60 per cent O_2 —1 to 2 days; 70 per cent O_2 —2 days; 80 per cent O_2 —3 to 4 days; 90 per cent O_2 —1 to 2 days; 100 per cent O_2 —as long as desired.

When the process of gradually increasing the oxygen in the atmosphere was completed, and a concentration of approximately 100 per cent was reached, control animals were placed in the chamber, in order to compare the mortality among such untreated animals with that among the "acclimatized."

In a few experiments a different method of "acclimatization" was tested. The animals were placed directly in 100 per cent oxygen, without any previous ex-

³ In some of the early experiments CO_2 concentration rose to nearly 1 per cent, and the O_2 content was therefore nearer to 99 per cent.

posure to lower concentrations, but the chamber was opened to atmospheric air from one to four times each day, for a total of 60 minutes a day. On re-closing the chamber each time it was flushed rapidly with oxygen for several minutes to ensure quick return to 100 per cent.

In one group of 10 rats observations were also made on the basal rate of consumption of oxygen before, during and after exposure to 100 per cent oxygen at a pressure of 1 atmosphere. The oxygen consumption was determined by means of a closed circuit apparatus, slightly modified from that described by Kaunitz and Pappenheimer (23). Records were made by a small spirometer of the Krogh type, with modifications introduced to obtain satisfactory counter-balancing in all positions of the float (24). An ink writing point was used, recording on the ordinary drum and paper of the Benedict-Roth basal metabolism apparatus. All determinations were carried out in 100 per cent oxygen, previous experiments having shown no difference in result between determinations in air and those in oxygen. If the animal had been living in air, it was placed in oxygen at least 1 hour before the record was made, in order to allow for equilibrium between the tissues and the gas. The usual duration of each record was 6 minutes, at least four curves being taken for each determination and only entirely linear records, indicating absolute quiescence of the animal, were used. The lowest value was taken as being that which most nearly represented true basal metabolism. Measurements were made at temperatures of 27 to 29°C. and the rats were weighed immediately after the test, the oxygen uptake being expressed in cubic centimeters per minute per kilogram of body weight.

In experiments on dogs oxygen was given in a chamber whose dimensions were $3\frac{1}{2} \times 1\frac{1}{2} \times 1\frac{1}{2}$ feet. The walls of the chamber were constructed of clear transparent vinylite mounted on a welded metal frame. Cooling was accomplished by incorporating the fluted wall of a metal ice container into one of the side walls of the chamber. Oxygen was run at a flow of 4 to 6 liters per minute which was sufficient to maintain the concentration above 98 per cent in all but one experiment in which a leak developed. Carbon dioxide was maintained below 0.6 per cent by drawing the atmosphere of the chamber through a soda-lime container. This was accomplished by means of an injector operated by the incoming oxygen.

The animals were usually treated in pairs, one receiving only oxygen while the other received oxygen and additional medication. When animals were removed from the chamber for blood sampling or medication they received oxygen by mask. The time of exposure to air, under these circumstances, never exceeded 3 minutes during any one removal. Arterial bloods were obtained from the femoral artery after novocainizing the site of puncture. Bloods were analysed for oxygen and carbon dioxide by the manometric method of Van Slyke and Neill. The pH was calculated by an adaptation of the method of Van Slyke. A log curve for carbon dioxide capacity was used to determine the $p\text{CO}_2$ and the CO_2 content at T_{37} .

RESULTS. Table 1 shows the mortality in rats exposed directly to an atmosphere of 100 per cent oxygen and in those acclimatized gradually by preliminary exposure to lower concentrations of oxygen rising by stages to 100 per cent. The

rats used were albino rats, usually males, from 4 to 6 months of age. In 100 control rats exposed continuously to 100 per cent oxygen the mortality in the first four days was 96 per cent, whereas in 69 rats previously acclimatized by stages the mortality during the same period was 7 per cent. Of the controls surviving

TABLE 1
Rat mortality in 100% oxygen

EXPERIMENT NO.	NO. RATS USED	NO. RATS DEAD EACH OF 1ST 4 DAYS:				NO. SURVIVORS AFTER 4 DAYS	AVERAGE LENGTH OF SURVIVAL
		1st day	2nd day	3rd day	4th day		
Control rats							
1 (a)	10	0	0	5	5	0	6 days 28 days
1 (b)	10	0	0	4	6	0	
4	15	0	0	10	5	0	
9	5	0	0	5	0	0	
10	5	0	0	3	2	0	
12	8	0	0	3	5	0	
13	2	0	0	1	1	0	
17	5	0	0	4	1	0	
B18 (a)	20	0	0	10	7	3	
B18 (b)	20	0	0	8	11	1	
Totals.....	100	0	0	53	43	4	Deaths in 4 days 96%

Acclimatized rats; Method I—Gradual increase in oxygen

4	15	0	0	0	0	15	20 days—9 sacrificed
12	10	1	0	0	0	9	19 days—1 sacrificed
12 (b)	5	1	0	0	0	4	7 days—all sacrificed
13	2	0	0	0	0	2	21 days
B14	12	2	0	0	0	10	40 days—8 sacrificed
B19	25	0	0	1	0	24	16 days—5 sacrificed
Totals.....	69	4	0	1	0	64	Deaths in 4 days 7%

EXPERIMENT NO.	OPENED TO AIR	NO. RATS USED	NO. RATS DEAD EACH DAY								SURVIVORS AFTER 8TH	AVERAGE TIME SURVIVAL
			1st	2nd	3rd	4th	5th	6th	7th	8th		

Method II—Periodic opening to air

19	Once—1 hr.	5	0	0	2	2	0	0	0	1 (in air)	0	
B17	4×—15 min.	20	0	0	0	0	0	3	1	2	14	20 days

more than 4 days only 1 lived for more than a week. The average length of life of the acclimatized rats surviving more than 4 days was 20 days. In most cases these animals were kept in 100 per cent oxygen for a week and then returned to room air, but in experiment B 19 they were kept in 100 per cent oxygen for 22 days.

The method of acclimatization by opening the chamber to room air for 1 hour

in each 24 was not successful, since 4 out of 5 rats died within 4 days. The single survivor was returned to room air and lived for 8 days. In a later experiment on 20 male rats, the chamber was opened for 15 minutes four times in each 24 hours, which resulted in a more successful acclimatization. During the first five days of this interrupted exposure to 100 per cent oxygen there were no deaths. Three rats died on the sixth day, one on the seventh and two on the eighth, while the remaining 14 rats survived for 20 days in 100 per cent oxygen with return to atmospheric air for 15 minutes four times a day (table 1, method II). They were then kept for 1 week in 100 per cent oxygen uninterruptedly without exposure to air except occasionally for provision of water and food. On the seventh day 3 rats died and the 11 survivors were returned to air.

In this last experiment, since the chamber was opened frequently, the opportunity was taken to weigh the rats once a day. As is common in acclimatized animals, even those that survive for many weeks in 100 per cent oxygen, loss of weight and of body fat was conspicuous. The average weight of the original 20 rats was 268 grams. During the first 12 days of acclimatization by periodic

TABLE 2

EXPERIMENT NO.	NO. OF RATS	PREVIOUS TREATMENT	TIME IN 100% O ₂	SERUM CHLORIDE		CO ₂	
				mgm. %	m.eq./l.	vols. %	m.eq./l.
4	8	Acclimatized to O ₂	12 days	556	95.1	81.6	36.5
B19	4	Acclimatized to O ₂	22 days	532	91.0	84.6	35.8
Normal rat	1	None	None	612	104.6	23.9	10.7

opening of the chamber, the intake of both water and food was markedly reduced, and the average weight of the survivors fell to 212 grams. During the last week of acclimatization there was no further loss of weight, but during the week of continuous exposure to 100 per cent oxygen the body weights decreased once more, falling to an average of 189 grams per rat. On returning to air, the survivors regained some weight though they did not reach their original weights during the period of observation.

In two other experiments, determinations of chloride content and carbon dioxide content were made on the pooled heart blood of a number of acclimatized rats that had survived for twelve and twenty-two days in 100 per cent oxygen. As shown in table 2, in both cases the serum chloride content showed a marked fall, and the carbon dioxide content an elevation, as compared with the values obtained in the normal animal.

The lungs of control rats dying about the third or fourth day of exposure to 100 per cent oxygen showed the characteristic intense congestion and edema with a large pleural effusion. The alveoli and sometimes the bronchi were filled with fluid and red blood corpuscles. In the acclimatized rats the lungs of those that died during the early days in 100 per cent oxygen, in whom acclimatization may be presumed to be lacking, showed very similar congestion, edema, pleural effusion, and often hemorrhage. After about the fourteenth day in 100 per cent

oxygen the edema was diminished, areas of atelectasis were developed between markedly distended alveoli, and an increased cellularity of the alveolar wall was observed. The amount of pleural fluid was usually diminished. After about three weeks in 100 per cent oxygen, the acclimatized lung usually showed no pulmonary edema or pleural fluid. The picture was that of a chronic organizing pneumonitis, with thickening of the cells of the alveolar walls, large areas of atelectasis, scattered areas of edema of slight degree and lymphocytic infiltration.

The duration of the increased tolerance to inhalation of 100 per cent oxygen was also investigated. Two rats that had been previously acclimatized, and had lived in 100 per cent oxygen for two weeks, were placed two months later in a chamber with 100 per cent oxygen. Both animals were alive after nineteen days (expt. 18—group C). Two other acclimatized rats that had been back in room air for eighteen days remained alive in a chamber with 100 per cent oxygen for twenty-one days (expt. 13). A third group of 10 acclimatized rats were kept in room air for two weeks and then returned to 100 per cent oxygen. They were all alive after twelve days in 100 per cent oxygen and were removed to air; whereas 9 out of 10 control rats, placed directly in 100 per cent oxygen died within a few days (expt. B 14).

In a previous attempt to acclimatize *rabbits* to 100 per cent oxygen, no acquired tolerance was observed by Barach (7). A further attempt to use the procedure here described was made in other species, but still without success. In thirteen mice, ten guinea pigs and five dogs⁴ no significant resistance to oxygen poisoning was demonstrated after gradual increase in the oxygen concentration of the atmosphere.

Because of the fact that carbon dioxide is said to increase the toxic effect of oxygen under increased pressures, the effect of 0.6 gram sodium bicarbonate and 0.2 gram sodium bicarbonate daily was tried in two groups of ten rats each. In another series sodium lactate was given in 1/6th molar solution daily, 10 cc. to seventeen rats and 20 cc. to eighteen rats, during exposure to 100 per cent oxygen. Comparison with an equal number of control animals showed no increase in duration of survival in 100 per cent oxygen with either bicarbonate or lactate.

In order to test the effect of a high carbon dioxide content with 100 per cent oxygen at 1 atmosphere of pressure the survival time of 20 rats in a chamber containing about 99.6 per cent oxygen and 0.3 per cent carbon dioxide was compared with that of 20 similar rats in a chamber containing approximately 98 per cent oxygen and 2 per cent carbon dioxide. As shown in table 3 no evidence was obtained of an increased toxicity with carbon dioxide at atmospheric pressure.

Measurement of the oxygen consumption in a group of 10 acclimatized rats showed a fall in oxygen uptake, averaging 12 per cent, in 9 of the animals. Approximately the same fall was shown whether the exposure to oxygen was 1, 2, 3, 4 or 5 days. The tenth rat showed no change in oxygen consumption. After 1 week back in air the oxygen uptake in 7 rats rose to nearly the pre-oxygen level, in 2 others it remained low, and in the tenth exceptional rat, which had shown no

⁴ Three of these dogs were the animals included in table 7, group III.

fall in oxygen consumption, there was actually a slight fall on return to air. Average values are shown in table 4.

Five control rats, placed directly in 100 per cent oxygen, showed no change in oxygen consumption during the first 24 hours. Within 48 hours there was a decrease of 7 per cent and in three days the fall was approximately 23 per cent. All the rats died within a few days (table 5).

TABLE 3

DAY	CHAMBER I (LOW CO ₂)		CHAMBER II (HIGH CO ₂)	
	Deaths	Survivals	Deaths	Survivals
3	11	9	11	9
4	6	3	8	1
5	0	3	0	1
6	3	0	0	1

TABLE 4

Changes in oxygen consumption and body weight during exposure to 100% oxygen
Average values for 10 acclimatized rats

	NORMAL IN AIR	AFTER ACCLIMATIZATION		PERCENTAGE FALL IN O ₂
		1-5 days in 100% O ₂	1 week in air	
				%
O ₂ consumption.....	18.9 cc.	16.9 cc.	17.9 cc.	11.9
Body weight.....	213 grams	189 grams	197 grams	11.3

TABLE 5

Changes in oxygen consumption and body weight during exposure to 100% oxygen
Average values for 5 control rats

	NORMAL IN AIR		IN 100% OXYGEN						% CHANGE IN 100% O ₂	
			1 day		2 days		3 days			
	O ₂ con.	Wt.	O ₂ con.	Wt.	O ₂ con.	Wt.	O ₂ con.	Wt.	O ₂ con.	Wt.
	cc.	g.	cc.	g.	cc.	g.	cc.	g.		
Rat 1.....	17.72	246	17.71	262					None	6.5% rise
Rats 2 & 3.....	17.88	235			16.6	256			7 % fall	9% rise
Rats 4 & 5.....	19.45	304					14.85	292	23.5% fall	4% fall

As always occurs, all the acclimatized rats lost weight, the average loss being about 11 per cent. After return to atmospheric air, most of the animals regained weight, though not reaching their original level, or attaining a normal growth curve. The question, therefore, arose whether the changes in oxygen consumption were merely due to the changes in body weight. Comparison of the oxygen consumption in normal (fed) and in starved rats showed that a weight loss of 13 per cent was accompanied by a fall in oxygen consumption of 17 per cent. Re-

covery of weight on feeding was accompanied by a rise in oxygen consumption (table 6).

It may be noteworthy that in the control rats body weight continued the normal growth curve for two days in 100 per cent oxygen, but the oxygen uptake

TABLE 6

Changes in oxygen consumption and body weight during exposure to 100% oxygen
Average values for 4 starved rats

	FED	STARVED	FED	% CHANGE IN STARVATION
				%
O ₂ consumption.....	17.7 cc.	14.68 cc.	17.7 cc.	17 fall
Body weight.....	251 grams	219 grams	259 grams	13 fall

TABLE 7

Survival time of dogs exposed to high oxygen concentrations

GROUP	ANIMAL NUMBER	%O ₂ IN INSPIRED AIR	SURVIVAL TIME IN HOURS	REMARKS
I	1	98+	116	Aborted first day—vomited throughout experiment
	2	98+	62	
	4	98+	83	
	5†	75-95	240	
II	11	98+	58	Given 330 cc. sodium lactate* during exposure
	12	98+	71	Given 460 mgm. Nembutal in divided doses each day of exposure
	13	98+	50	Given 600 cc. sodium lactate, 200 cc. 5% glucose in saline and 100 cc. dog plasma during exposure
	14	98+	50	Given 160 cc. sodium lactate, 130 cc. dog plasma and 400 cc. 5% glucose in saline during exposure
	15†	75-95	270	Given 780 cc. sodium lactate during first four days of exposure
III	23	98+	48	Received 50% oxygen for 3 days, 60% oxygen for 4 days, 70% oxygen for 3.5 days prior to exposure to 98+% oxygen
	26	98+	72	Received 50% oxygen for 2 days, 60% for 1.5 days, 70% for 1.5 days, 80% for 2 days, 90% for 1 day prior to exposure to 98+% oxygen
	27	98+	168	Same as no. 26

* Sodium lactate was administered subcutaneously in 1/6 molar solution.

† Leak in chamber caused fluctuation in oxygen concentrations between 75% and 95% throughout the experiment.

showed a drop of 7 per cent between the first and second day. By the end of the third day a slight loss of weight (4 per cent) had begun, but a very marked and rapidly increasing fall (23 per cent) in oxygen uptake had occurred (table 5). Since, therefore, the decrease in oxygen consumption appears to precede the loss

of weight, it is possible there may be some additional cause beside the decrease in weight for the lowering of the oxygen consumption during oxygen poisoning.

The survival time of dogs in high oxygen concentrations is shown in table 7. Twelve dogs were used in these experiments. They were treated in pairs as follows: no. 1 and no. 11, no. 2 and no. 12, no. 13 and no. 23, etc. The animals were divided into 3 groups: I, oxygen control group; II, medicated group; and

TABLE 8

Arterial blood gases, pH, pCO₂ and chlorides of dogs exposed to high oxygen concentrations

DOG NO.	HOURS OF EX-POSURE IN O ₂	% O ₂ IN IN-SPIRED AIR	O ₂ CONT.	O ₂ CAP.	% O ₂ SAT.	CO ₂ CONT.	pCO ₂	pH	Chlorides	CO ₂ CONT. T40	REMARKS
			vol. %	vol. %		vol. %			mg. %		
1	0	21	18.3	19.2	95.3	37.2	29.3	7.44		42.1	Ill, aborted 1 day in oxygen Vomited throughout experiment
	18	98+	16.1	15.2	100+	38.0	35.7	7.33	618	39.6	
	42	98+	15	14.1	100+	45.8	41.5	7.34		45.3	
	66	98+	13.8	13.2	100+	50.7	42.9	7.37	584.3	49.7	
	90	98+	13.1	12.1	100+	52.3	49.2	7.30		49.2	
	114	98+	12.1	12.2	99.2	55.2	64.5	7.21		47.6	Died 116 hrs.
5	0	21	13.4	14.4	93.1	50.9	36.8	7.45	602	51.7	Died 240 hrs.
	24	72-85	17.2	14.7	100+	46.3	43.3	7.32	572	45.0	
	48	72-85	17.8	16.9	100+	46.0	45.1	7.31	560	43.9	
	72	85-90	17.2	16.1	100+	45.6	34.1	7.44	554	48.4	
	96	85-90	15.3	13.2	100+	52.6	36.7	7.46	593	53.9	
	120	85-90	12.9	11.2	100+	50.7	38.1	7.42	582	51.5	
	144	98+	12.1	10.5	100+	54.0	41.8	7.39		53.4	
	192	98+	12.7	11.8	100+	58.8	63.2	7.25	575	60.6	
23	0	21	20.0	20.4	98.1	39.0	30.6	7.44	578	43.6	Died after 48 hrs. in 98+% O ₂
	0	21	18.3	18.5	99	42.6	34.0	7.43		45.3	
	72	50	19.7	18.1	100+	46.7	37.8	7.41	574	47.7	
	86	60	16.0	15.4	100+	54.4	42.2	7.42	618	53.6	
	72	70	13.8	12.9	100+	46.0	31.3	7.47	641	51.6	
	46	98+	15.4	15.1	100+	42.6	41.2	7.33	607	52.1	

III, acclimatized group. The average survival time for group I in 98+ per cent oxygen was 87 hours. We are not including dog 5 in this average because he received only 75 to 95 per cent oxygen throughout the experiment. The animals in group II, the medicated group, survived for an average of 56 hours in 98+ per cent oxygen. Dog 15 is not included in this average. The acclimatized group who were treated with oxygen concentrations increasing from 50 per cent to 80 per cent over an 8 to 10 day period survived for an average of 96 hours after being placed in 98+ per cent oxygen.

The data obtained from analysis of the blood gases appear in table 8. We are omitting the values for animals in group II because the medication given the animals tended to retard some of the changes noted in the control and acclimatized group. The overall blood picture of the medicated animals, however, was very similar to that shown for dogs 1, 5 and 23 in the table. The changes may be summarized as follows: There was a consistent decrease in oxygen capacity in all animals. The percentage oxygen saturation remained elevated even during the last days of survival. No attempt was made to calculate the exact hemoglobin saturation while the animals were breathing oxygen since the quantity of oxygen dissolved in the plasma under these circumstances could only be approximated. The values for oxygen content in the table include both the oxygen combined with hemoglobin and that dissolved in the plasma. The CO_2 content and pCO_2 rose throughout the exposure to oxygen even when the oxygen percentage breathed by the animals was less than 98+ per cent. There was a fall in pH in all cases which became marked during the last day of survival. The carbon dioxide capacity at T_{40} rose in every case indicating an increase in alkali reserve. The blood chlorides fluctuated but in five out of nine instances there was a significant decrease.

DISCUSSION. The effect of gradually increasing the oxygen concentration in the inspired air from 60 per cent to 100 per cent is to prolong the duration of life in rats exposed to 100 per cent oxygen and to prevent death from acute pulmonary edema. In addition, animals that survive a period of ten to twenty days in 100 per cent oxygen do not show the large collection of pleural fluid which is seen in control animals that die between the third and fourth day of exposure to 100 per cent oxygen. Since a partial tolerance to 100 per cent oxygen may be built up within a period of six to twelve days, the development of resistance of oxygen poisoning cannot be accounted for on the basis of chronic changes in pulmonary epithelium which are subsequently found. However, the increased tolerance changes manifested by animals three weeks to two months after acclimatization may be accounted for in part by thickening of the alveolar walls. Exposure to a gradually increasing concentration of oxygen does result in some irritation to the pulmonary epithelium with scattered slight edema but a mechanism as yet unknown develops which prevents the animal from being overwhelmed by the profuse pulmonary edema and pleural effusion which takes place in control animals placed in 100 per cent oxygen. The attempt to modify the phenomenon of oxygen poisoning by administration of sodium bicarbonate or sodium lactate was unsuccessful.

From the pathologic picture in the lungs, viz., thickening of the alveolar walls with scattered microscopic edema, it is evident that the diffusion of oxygen into the alveoli was markedly impaired. This was further confirmed by the dyspnea which was generally manifested when these animals were removed from the chamber. Elevation of the carbon dioxide content in an atmosphere of pure oxygen, together with the fall in serum chlorides, is similar to the mechanism referred to above in human patients with chronic pulmonary disease treated with 50 per cent oxygen (22, 25). Barach and Woodwell (26) described in patients with shallow breathing, suffering from lethargic encephalitis, a similar marked rise in carbon

dioxide content to 88 vol. per cent, when these patients were treated with 100 per cent oxygen for two hours. Oxygen inhalation makes possible a lower pulmonary ventilation, which is followed by an increased content and tension of carbon dioxide in the arterial blood and an increase in carbon dioxide concentration in the expired air, with the result that the elimination of carbon dioxide is maintained at a lower volume of breathing. The fall in chlorides is a compensatory reaction to the increased carbon dioxide tension.

When acclimatized rats that showed an increased resistance to pure oxygen at sea level were exposed to oxygen under a pressure of fifty-five pounds per square inch, no increased tolerance to oxygen under pressure was observed by a study of mortality rates in this group as compared to a control series.

Whether changes in the oxygen enzyme system take place in six to twelve days during acclimatization to increasing oxygen concentrations cannot be decided from these studies. That rabbits and guinea pigs do not appear to develop an increased resistance to oxygen poisoning was at first ascribed to their being herbivorous animals. The dog experiments also were negative, but were too few in number to be considered decisive.

It was also evident that fifteen minutes' exposure to air four times a day resulted in a marked increase in survival in 100 per cent oxygen. This has a bearing on the clinical use of 100 per cent oxygen by mask, in which interruption of treatment for feeding and other purposes is generally employed.

The rise in carbon dioxide content of heart blood to 83 vol. per cent with a fall in chlorides from 612 to 556 mgm. per cent, which took place on the twelfth day of residence in 100 per cent oxygen in previously acclimatized rats, is a pathophysiological event which has been previously described in patients with pulmonary emphysema and fibrosis treated with 50 per cent oxygen. The swollen alveolar walls, with scattered areas of congestion and edema, impaired the diffusion of oxygen even in the presence of 100 per cent oxygen. The inhalation of 100 per cent oxygen in the presence of this degree of pulmonary pathology probably was instrumental in lowering the volume of breathing to such an extent as to result in an increased carbon dioxide content and a compensatory elimination of chlorides.

SUMMARY AND CONCLUSIONS

1. Methods are described for increasing the resistance of animals to oxygen poisoning: a, by a gradual increase in the concentration of oxygen from 60 per cent to approximately 100 per cent; b, by direct exposure to 100 per cent oxygen, but with return to atmospheric air four times daily for a total of 60 minutes a day.

2. Acclimatization of rats by the first method decreased the death rate for the first four days in 100 per cent oxygen from 96 per cent with the control rats to 7 per cent with acclimatized, with an average survival time of 20 days for the remaining acclimatized animals (93 per cent).

3. Acclimatization of rats by the second method showed a mortality of 33 per cent within 8 days, and a survival time of 27 days for the remaining 67 per cent, as compared with a 99 per cent mortality within 8 days for control rats.

4. Dogs treated by the first method showed at most a negligible increase in

survival time from 87 hours for control animals to 96 hours for "acclimatized." Mice, rabbits and guinea pigs showed no increase of resistance to 100 per cent oxygen by this method.

5. Persistence for periods up to 2 months of this acquired tolerance to oxygen in rats was shown. This late manifestation of acquired tolerance may be related to the increased cellularity and thickness of the alveolar wall which is seen in such acclimatized animals, although the resistance to the first exposure to 100 per cent oxygen is not of this nature.

6. The presence of increased amounts of CO_2 in the atmosphere did not increase the toxicity of oxygen at a pressure of approximately 1 atmosphere.

7. Administration of sodium bicarbonate or of sodium lactate did not increase the time of survival of either rats or dogs.

8. In dogs exposed to 100 per cent oxygen there was a progressive fall in oxygen capacity of the blood, a rise in the arterial CO_2 content and pCO_2 and a fall in pH. The rise in the alkali reserve of the blood and the fall in blood chlorides are probably associated with a homeostatic mechanism previously described, but these alterations are insufficient to maintain a normal pH. Similar changes in the CO_2 content and blood chloride levels were found in rats exposed to the same conditions, namely, a lowered serum chloride content which is a compensatory reaction to an elevated CO_2 tension. The diminished volume of ventilation which inhalation of oxygen engenders in the presence of damaged pulmonary epithelium is followed by a progressive rise in CO_2 content and pressure. The latter makes possible elimination of CO_2 with a smaller pulmonary ventilation.

9. The oxygen consumption in rats fell during acclimatization, but less markedly than in control rats placed directly in 100 per cent oxygen. It appeared uncertain whether this fall should be regarded as due merely to the concomitant loss of weight.

10. The practical value of these studies is chiefly the increase in resistance to the effects of inhalation of 100 per cent oxygen which is obtained by intermittent 15 minute exposure to breathing air. The mechanism by which acquired tolerance to inhalation of pure oxygen is obtained has not been revealed by these studies.

REFERENCES

- (1) BERT, P. *La pression barométrique. Recherches de physiologie expérimentale*, Paris, Masson, 1878.
- (2) SMITH, J. L. *J. Physiol.* 24: 19, 1899.
- (3) HILL, L. *Quart. J. Exper. Physiol.* 23: 49, 1933.
- (4) KARSNER, H. T. *J. Exper. Med.* 23: 149, 1916.
- (5) STADIE, W. C., B. C. RIGGS AND N. HAUGAARD. *Am. J. Med. Sc.* 207: 84, 1944.
- (6) BINGER, C. A. L., J. M. FAULKNER AND R. L. MOORE. *J. Exper. Med.* 45: 849, 1927.
- (7) BARACH, A. L. *Am. Rev. Tuberc.* 13: 293, 1926.
- (8) SMITH, F. J. C., J. W. HEIM, R. M. THOMSON AND C. K. DRINKER. *J. Exper. Med.* 56: 63, 1932.
- (9) SMITH, F. S. C., G. A. BENNETT, J. W. HEIM, R. M. THOMSON AND C. K. DRINKER. *J. Exper. Med.* 56: 79, 1932.
- (10) HEDERER, C. AND L. ANDRE. *Bull. Acad. d. méd.* 123: 291, 1910.

- (11) DE ALMEIDA, A. O. *Compt. rend. Soc. de biol.* **116**: 1225, 1934.
- (12) SAYERS, R. R. Personal communication.
- (13) BARACH, A. L. AND M. SOROKA. Unpublished observations.
- (14) PAINE, J. R., D. LYNN AND A. KEYS. *J. Thoracic Surg.* **11**: 151, 1941.
- (15) GESELL, R. *This Journal* **66**: 5, 1923.
- (16) BEHNKE, A. R., L. A. SHAW, C. W. SHILLING, R. M. THOMSON AND A. C. MESSER.
This Journal **107**: 13, 1934.
- (17) CAMPBELL, J. A. *J. Physiol.* **63**: 325, 1927.
- (18) CAMPBELL, J. A. *J. Physiol.* **68**: 7, 1929.
- (19) EVANS, J. H. *New York State J. Med.* **39**: 709, 1939.
- (20) BOOTHBY, W. M., C. W. MAYO AND W. R. LOVELACE, JR. *J. A. M. A.* **113**: 477, 1939.
- (21) BECKER-FREYSANG, H. AND H. G. CLAMANN. *Klin. Wehnschr.* **18**: 1382, 1939.
- (22) BARACH, A. L. AND D. W. RICHARDS. *Arch. Int. Med.* **48**: 325, 1931.
- (23) KAUNITZ, H. AND A. PAPPENHEIMER. *This Journal* **138**: 328, 1943.
- (24) NICKERSON, J. L. B. G. KING AND H. J. CURTIS. *Rev. Scientific Instruments* **15**: 12,
1944.
- (25) RICHARDS, D. W., JR. AND A. L. BARACH. *Quart. J. Med.* **3**: 437, 1934.
- (26) BARACH, A. L. AND M. N. WOODWELL. *Arch. Int. Med.* **28**: 421, 1921.

EVIDENCE THAT THE PHYSIOLOGIC NORMAL HEMOGLOBIN VALUE FOR ADULT DOG BLOOD IS 18 GRAMS PER 100 CC.

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Previous work in this laboratory (1) has shown that the hemoglobin of healthy, adequately fed control dogs reaches a level of 18 grams per 100 cc. of blood, a value much higher than that commonly accepted as normal, namely, 14 grams. The latter figure apparently is based on the work of Scarborough (2), who collected from the literature blood values on many commonly used laboratory animals, including the dog, which were "incidental to or for the control of experimental work in which alterations of the picture were expected." No attempt was made to include data later than 1926, whereas much evidence has accumulated since then to indicate that the B-complex vitamins play an important rôle in blood formation (3-11).

It is well known that in most animal species there is a progressive increase in the hemoglobin content of the blood from the weanling period to the permanent adult level. It would seem logical, then, in seeking the adult value to follow this progress at short intervals throughout the period of growth into the adult phase of life while the animal subsists on a diet adequate, according to present knowledge, in all blood-building factors. Many investigators have shown that vitamin B-complex factors, in addition to the commercially available synthetic ones, are important for blood formation (8, 11); consequently, in choosing the diet, care must be taken to include all these factors, along with adequate protein and mineral elements known to be required for hemoglobin production. This has been done with entirely consistent results in ten puppies.

EXPERIMENTAL. In the selection of our experimentally adequate diet, care has been taken to include in liberal amounts all those factors known to be required for blood formation, namely, protein, a mineral salt with the essential trace elements and a natural source of the vitamin B-complex, thus providing the unknown as well as the known factors. The following diets have been employed in this study:

1. *Yeast diet:* Casein, 40 per cent; sucrose, 26; cotton seed oil, 18; cod liver oil, 2; mineral salt,¹ 4; dried brewer's yeast, 10 per cent.

2. *Basic (B-complex free) diet:* Identical with the above yeast diet except that the yeast was replaced by sucrose.

¹ Mineral salt mixture: Bone meal (steamed), 57.8 per cent; sodium chloride, 24.4 per cent; lime stone (oyster shell flour), 12.2 per cent; iron sulfate (U. S. P.), 3.7 per cent; magnesium oxide (U. S. P.), 1.2 per cent; copper sulfate (reagent), 0.3 per cent; manganese sulfate (reagent), 0.1 per cent; zinc oxide (reagent), 0.1 per cent; cobalt carbonate, 0.1 per cent; potassium iodide, 0.1 per cent.

3. *Stock diet*: Bread, 53 per cent; red meat, 41.3; cow peas, 4.7; sodium chloride, 1 per cent.

Some of the animals received, in addition, eight synthetic vitamin supplements, namely, thiamin hydrochloride, 1.4 mgm.; riboflavin, 0.7 mgm.; pyridoxine, 6 mgm.; nicotinic acid, 6 mgm.; inositol, 6 mgm.; pantothenic acid, 6 mgm.; para-aminobenzoic acid, 6 mgm.; and choline, 30 mgm. per dog per day.

The following technique was used for blood determinations: All quantitative measurements were made on blood drawn from the jugular vein into tubes containing potassium oxalate (2 mgm. per cc. of blood). Hemoglobin was determined in most instances by means of a Sahli hemoglobinometer, standardized by checking against bloods of known hemoglobin concentration as determined by the Van Slyke method. Later, to eliminate the human factor in matching color, a Fisher Electrohemometer was used. There was no significant difference in the values obtained by the two methods.

Each of the eighty animals used in these experiments was housed in an individual cage and given access to food and water at all times. In addition, the puppies were freed from intestinal parasites, immunized against distemper and observed to have normal appetite and growth rates before being placed on experiment.

Group I, consisting of ten healthy puppies from different litters with an initial age of from eight to twelve weeks, was placed on the yeast diet. Four of these animals received, in addition, the eight synthetic vitamins in the amounts indicated above.

All the animals continued to grow at a normal rate and showed a slow, progressive rise in hemoglobin from an average initial value of 10.5 grams per 100 cc. of blood (between the ages of 8 and 12 wks.) to an average of 18.8 grams after about fifteen weeks on the diet, when the adult plateau in the hemoglobin curve was reached (fig. 1 A and B). This average was derived from 130 hemoglobin determinations on the ten puppies after they had reached the adult level while still subsisting on the yeast diet. Although the hemoglobin of puppies receiving the synthetic vitamin supplement tended to reach somewhat higher levels, they are included in the average and, consequently, tend to raise it slightly (fig. 1 B). During the period of increase in hemoglobin values, corresponding increases in red blood count and hematocrit were observed. These increases occurred without appreciable reticulocytosis. The red blood count rose from an average of 4,620,000 to 7,590,000, while the hematocrit increased from an initial value of 34.4 to 54.1 volumes per cent.

Group II consisted of two adult dogs. This experiment was designed to exclude the possibility that the high hemoglobin values obtained in group I were due to the tendency of young animals to go beyond the normal hemoglobin value only to return later to a somewhat lower value. Both of these adult dogs had initial hemoglobin values of 16 grams. After they were placed on the yeast diet, their hemoglobin levels rose very gradually to 18 grams, where they remained with slight variations throughout the entire experimental period of eighty-five weeks. The weight and hemoglobin changes of one of these animals are typical (fig. 1 C).

The high values obtained in group I might be challenged as being due to polycythemia caused by an extraneous toxic factor such as cobalt or to dehydration. The experiments performed in groups III and IV were designed to answer these two questions respectively.

Group III consisted of three adult dogs treated with cobalt while subsisting on the stock diet. Each dog was given orally 15 mgm. of cobalt per kilogram per day (as $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$), the lowest level found by Brewer (12) to result in elevated hemoglobin, red blood count and hematocrit values. The peak in the blood

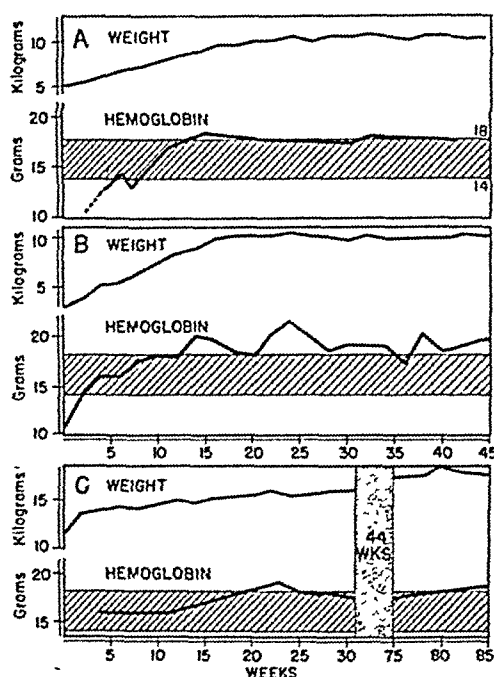


Fig. 1

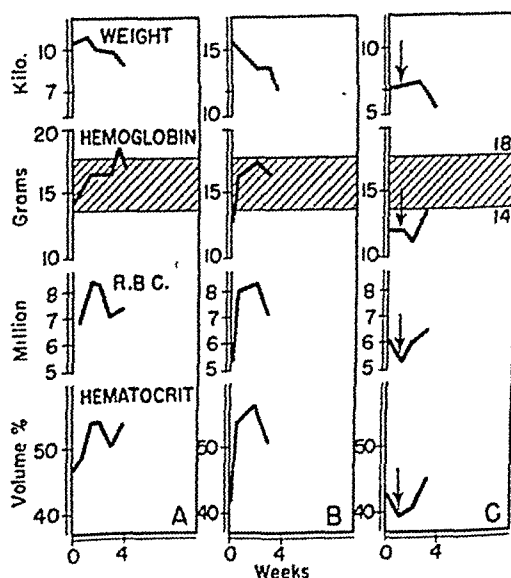


Fig. 2

Fig. 1. Weight and hemoglobin curves of dogs receiving yeast at a level of 10 per cent in the diet. A. Puppy (initial age 8 wks.) receiving yeast as the sole source of the vitamin B-complex. A determination was not made at 10 weeks, so the averaged value is used for the point. B. Puppy (initial age 11 wks.) receiving yeast plus V (8 synthetic vitamins listed in text) as its source of the vitamin B-complex. C. Adult (age unknown) receiving yeast as the sole source of the vitamin B-complex.

Fig. 2. Weight, hemoglobin, red blood count and hematocrit curves of dogs receiving cobalt (15 mgm./kilo orally). In A and B the dosing started at the beginning of the experimental period. The beginning of dosing in C is indicated by the arrow.

curve was reached usually within two weeks, after which there was a slight fall. The highest hemoglobin level reached by any of these dogs was 18.5 grams per 100 cc., while the dog with the lowest initial hemoglobin never reached 14 grams. Cobalt at this level proved quite toxic, and all the dogs lost weight fairly rapidly during the cobalt feeding. Two dogs died before the end of the experimental period of four weeks (fig. 2 B and C). The increased blood values observed here cannot be confused with the results obtained in group I. The sharp rise induced by the cobalt is probably a toxic effect, and the higher levels are not maintained

(fig. 2). Cobalt, if present in the yeast diet, presumably occurs in the brewer's yeast itself. An analysis (13) was made at the Fleischmann Laboratories through the courtesy of Dr. Charles N. Frey which indicated that the yeast of the type used in these experiments contained only 2.8 parts per million of cobalt, or 0.00028 per cent. This rules out the possibility that cobalt was present in the diet at toxic levels.

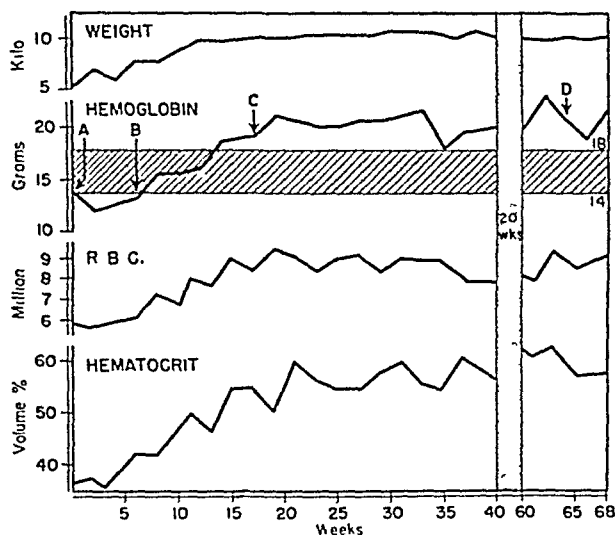


Fig. 3. Weight, hemoglobin, red blood count and hematocrit curves of a dog treated with yeast after previous depletion on a vitamin B-complex deficient diet. The letters *A*, *B*, *C* and *D* on the hemoglobin curve indicate the points at which the photographs in figure 4 were made.

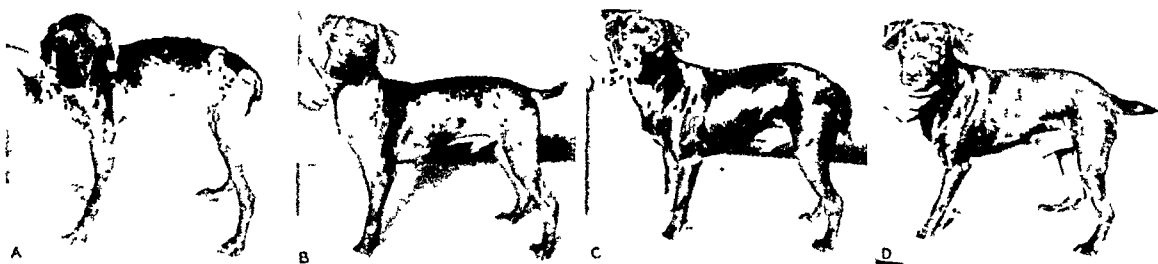


Fig. 4. Photographs of dog with blood factors charted in figure 3. *A*. Appearance when yeast treatment was begun. *B*. Appearance after 6 weeks of yeast ingestion. *C*. Appearance after 17 weeks of yeast ingestion. *D*. Appearance after 64 weeks of yeast ingestion.

Group IV consisted of five puppies placed first on the yeast diet to build them up physically and to allow their hemoglobin to reach a value of 14 grams. Then they were given the basic B-complex free diet, supplemented with the eight synthetic vitamins described above, to ascertain whether these synthetic factors alone were adequate as a source of the vitamin B-complex. Under these conditions, the hemoglobin showed a gradual rise up to, or slightly above, 18 grams, probably due to stored unknown factors of the B-complex necessary for blood formation which presumably became exhausted soon after this point was reached. The hemoglobin values then gradually decreased. Growth ceased entirely and

the animals became weak, emaciated and extremely dehydrated. In spite of this, their blood values continued to decrease, the hemoglobin falling below 14 grams in every case (10 to 13.5 grams). If the dogs were allowed to continue on the diet, the hemoglobin reached much lower levels and death finally ensued. Three animals of this group subsequently were placed on the yeast diet. Growth was resumed immediately and the general well-being of the dog improved, while the blood values continued to fall for a period of five to six weeks. This, we believe, was due to a hydration effect from the yeast (figs. 3 and 4). After the preliminary drop and after the hemoglobin had returned to the initial level, there was a consistent rise to values identical with those observed in the animals of group I (fig. 3).

Group V consisted of sixty apparently normal dogs chosen at random (50 stock dogs, 10 pets) on which hemoglobin and hematocrit determinations were made. Red and white cell counts also were done on about half of the animals. The hemoglobin values ranged from 10 to 22 grams per 100 cc. of blood, with an

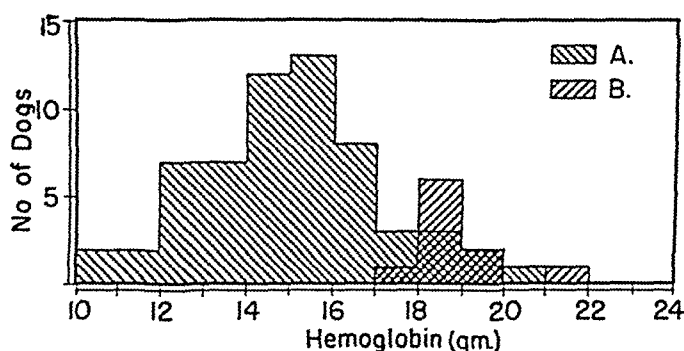


Fig. 5. Hemoglobin distribution curve. A. Dogs without special provision for an adequate source of the B-complex factors. B. Dogs receiving yeast at a level of 10 per cent as their source of the B-complex factors.

average of 15.1 grams. The ones with the higher hemoglobin values were definitely healthier.

Figure 5 shows the distribution of the hemoglobin values of the yeast-treated dogs as compared with that of dogs chosen at random without reference to nutritional background (stock dogs and pets). The charts are based on averaged hemoglobin values obtained in each of the ten yeast-treated dogs of group I after the adult level was reached (130 determinations) and similar averaged values obtained in each of the sixty other dogs (109 determinations). The distribution of the hemoglobin values of both groups appears to be normal. It is interesting, however, that in dogs with the additional B-complex supplied by the yeast the peak was between 18 and 19 grams as compared with a peak of between 15 and 16 in the other dogs.

DISCUSSION. Evidence has been presented which demonstrates that the physiologic normal value for hemoglobin in the adult dog is 18 grams or more per 100 cc. of blood. The normal value currently accepted by many investigators is between 13 and 14 grams (2, 14, 15). The most probable reason for the

difference between the values reported here and those reported previously is a difference in vitamin B-complex content of the diet. The present values were obtained by following the blood changes at intervals throughout the growth period and into adult life, while the animals consumed a diet adequate in the vitamin B-complex. The lower figure was obtained by the earlier investigators as a result of averaging a significantly large number of determinations by different investigators on adult dogs chosen without reference to nutritional status. The difference in amount of vitamin B-complex factors present in the diets could easily account for the difference in this figure (18 grams) and previous figures (13 and 14 grams). The dog is carnivorous and, if left to his own devices, kills his prey and consumes the entire carcass. Under these conditions he probably gets an adequate amount of the B-complex and essential minerals; but since his domestication and dependence on man, his diet has consisted too largely of muscle meat. Liver, as well as the other visceral organs, contains many of the B-complex factors not found in muscle. It is possible to elevate the hemoglobin of the dog to 18 grams or more under conditions similar to those described above when liver (hog) serves as the sole source of the B-complex (16). Yeast was used as our source of the B-complex because it has fewer extraneous factors.

Whether a dog's hemoglobin is 14 grams or 18 grams per 100 cc. apparently makes very little difference in his general well being. His health seems almost as good with a hemoglobin of 14 grams as 18 grams per 100 cc. of blood. However, in our experience, where the dog serves as his own control, there is a definite increase in vitality and general health as the hemoglobin increases. On the other hand, in the case of the experimental animal, particularly where he serves as subject for the assay of blood-forming factors, it is extremely important to know the actual physiologic normal. Without this information, accurate interpretation of data in blood studies is impossible.

SUMMARY AND CONCLUSIONS

Ten mongrel puppies from several litters were placed on a diet adequate, according to present nutritional standards, in all blood-building factors. Hematological examination of their blood was made at regular intervals throughout the growing period and into adult life. From an average initial value (8 to 12 wks. old) of 10.5 grams, the hemoglobin rose gradually in a period of approximately fifteen weeks to values averaging 18.8 grams. During this same time interval, the red blood count rose from 4,620,000 to 7,590,000 and the hematocrit from 34.4 to 54.1 volumes per cent.

These high blood values cannot be accounted for by dehydration or cobalt poisoning from the yeast. Analysis of the yeast used indicates that it contains only 2.8 parts per million of cobalt, or 0.00028 per cent.

The hemoglobin values in a series of sixty dogs, chosen without special provision for the B-complex factors in the diet, varied from 10 to 22 grams, averaging 15.1 grams per 100 cc. of blood.

It is concluded from this study that, under the conditions described, the physiologic normal value for hemoglobin of the adult dog blood is 18 grams per

cc. and that a red blood count of 7,590,000 and hematocrit reading of 54.1 are corresponding values.

Acknowledgments. We are indebted to the Lederle Laboratories, Pearl River, N. Y., for a grant in aid, for supplies of choline and inositol, for material with which the dogs were immunized against distemper and for the vermifuge administered; to Merck & Company, Rahway, N. J., for supplies of vitamin B₆ and pantothenic acid; and to the Fleischmann Laboratories, New York City, for the brewer's yeast used. The work was aided further by support from the Anna H. Hanes Research Fund and by a grant from the Duke University Research Council.

We also wish to thank Dr. William Moore, Jr., of Cary, N. C., for timely assistance.

REFERENCES

- (1) SMITH, S. G., R. CURRY AND H. HAWFIELD. *Science* 98: 520, 1943.
- (2) SCARBOROUGH, R. A. *Yale J. Biol. Med.* 3: 359, 1931.
- (3) RHOADS, C. P. AND D. K. MILLER. *J. Exper. Med.* 58: 585, 1933.
- (4) DAY, P. L., W. C. LANGSTON AND C. F. SHUKERS. *J. Nutrition* 9: 637, 1935.
- (5) CHICK, H., T. F. MACRAE, A. J. P. MARTIN AND C. J. MARTIN. *Biochem. J.* 32: 2207, 1938.
- (6) FOUTS, P. J., O. M. HELMER, S. LEPKOVSKY AND T. H. JUKES. *J. Nutrition* 16: 197, 1938.
- (7) SMITH, S. G., R. REISER AND G. T. HARRELL. *J. Clin. Investigation* 20: 369, 1941.
- (8) SIMMONS, R. W. AND E. R. NORRIS. *J. Biol. Chem.* 140: 679, 1941.
- (9) HANDLER, P. AND W. P. FEATHERSTON. *J. Biol. Chem.* 151: 395, 1943.
- (10) WINTROBE, M. M., R. H. FOLLIS, JR., M. H. MILLER, H. J. STEIN, R. ALCAYAGA, S. HUMPHREYS, A. SUKSTA AND G. E. CARTWRIGHT. *Bull. Johns Hopkins Hosp.* 72: 1, 1943.
- (11) DAFT, F. S. AND W. H. SEBRELL. *Pub. Health Repto., U.S.P.H.S.* 58: 1542, 1943.
- (12) BREWER, G. *This Journal* 128: 345, 1940.
- (13) PARKS, R. Q., S. L. HOOD, C. HURWITZ AND G. H. ELLIS. *Ind. and Eng. Chem. Anal. ed.* 15: 527, 1943.
- (14) DUKES, H. H. *The physiology of domestic animals.* Ithaca, N. Y. 5th ed. (1943), p. 33.
- (15) HAYDEN, C. E. *The Cornell Veterinarian* 33: 85, 1943.
- (16) SMITH, S. G. Unpublished data.

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BLOOD pH DURING DECOMPRESSION¹

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The change in the reaction of the circulating arterial blood produced by rapid variation in the pressure of the ambient atmosphere has been studied in anesthetized dogs.

METHODS. Dogs anesthetized with nembutal were placed in a small decompression chamber. Sufficient "chlorazol fast pink" was injected intravenously to render the blood incoagulable. A glass electrode was placed in a femoral artery and a continuous record of the blood pH was obtained by methods already described (Nims, 1938). The respiratory rate was determined by observation through a window in the decompression chamber.

Arrangements were made so that either air or oxygen could be supplied to the animal at the pressure within the chamber without significantly altering that pressure, the total pressure within the chamber being under independent control. Repeated decompressions at varying rates were done on each animal.

RESULTS. A. *Decompression in air.* When the air pressure is rapidly reduced the pH of the arterial blood changes in an alkaline direction. The shift in reaction of the blood begins almost as soon as the reduction of the air pressure. The respiratory rate does not increase markedly with reduced pressure until the pressure is quite low. If the exposure to reduced air pressures is not too prolonged the effect on the blood is completely reversible. Figure 1 gives the results of such an experiment.

B. *Decompression in oxygen.* As might be expected decompression in oxygen to a pressure of 180 mm. Hg does not give rise to marked changes in either the blood reaction or in the respiratory rate. The slight increase in respiration and the small alkaline shift (0.03 pH unit) recorded in figure 2 probably indicate that the means of supplying oxygen was slightly inadequate.

C. *Sudden anoxia.* When an animal is maintained in pure oxygen at reduced pressure and anoxia is suddenly produced by administration of air at this pressure there is a prompt and rapid shift of the arterial blood pH in an alkaline

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direction accompanied by a rise in the frequency of breathing. Restoration of the oxygen restores both the rate of respiration and the blood reaction to pre-anoxic levels. In some experiments the reaction of the arterial blood may overshoot in an acid direction before leveling off as is demonstrated by figure 3.

D. Prolonged anoxia. As the above experiments demonstrate, the response of the animal to a reduced partial pressure of oxygen is a shift in the reaction

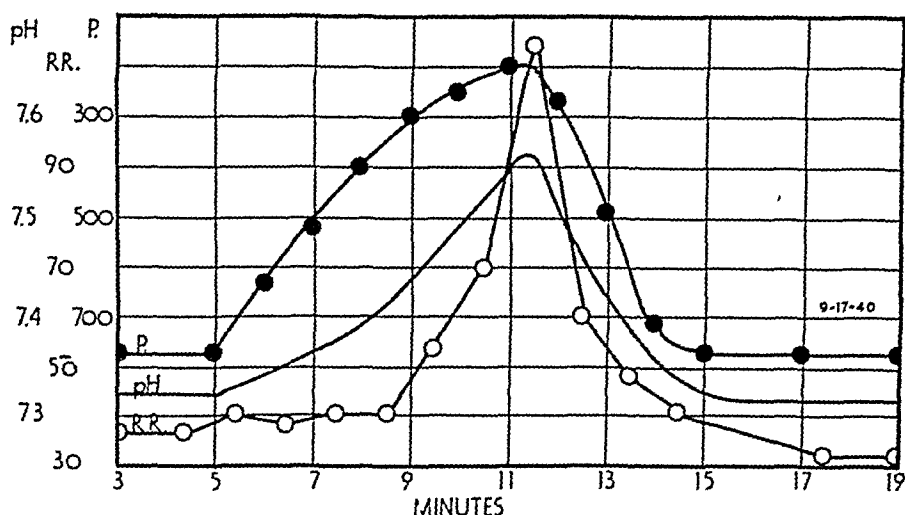


Fig. 1. Decompression in air. Dog, nembutal anesthesia. pH, —. Respiratory rate, R.R., — ○ —. Pressure, — ● —. Note marked alkaline swing of the arterial blood.

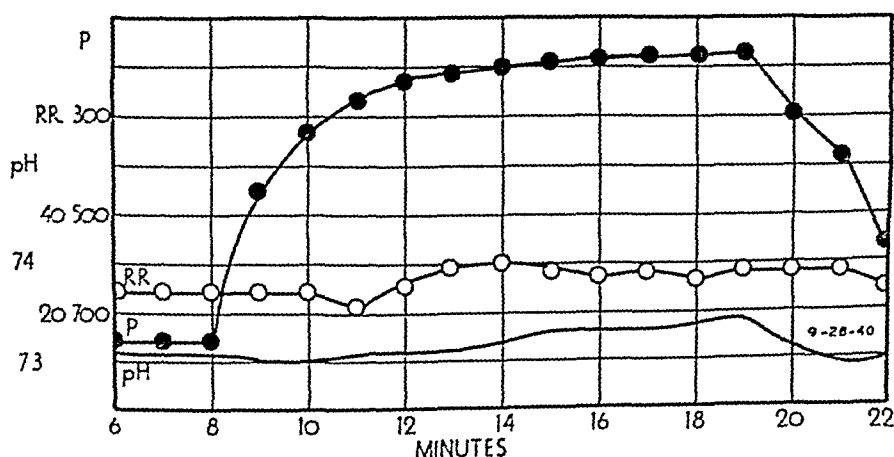


Fig. 2. Decompression in oxygen. Dog, nembutal anesthesia. pH, —. Respiratory rate, R.R., — ○ —. Pressure, — ● —. Note constancy of blood pH.

of his arterial blood to more alkaline levels. If the partial pressure of oxygen becomes too low or the exposure to the low pressure is too prolonged the animal can no longer maintain this alkaline reaction and the pH of the blood may then begin to shift in an acid direction. This turning point in blood reaction is a sensitive indicator of the extreme physiological limits of the animal for if the experiment is continued for a short period after the turning point is reached the

animal fails rapidly and soon ceases to breathe. If the anoxia is relieved either by decompression or by the administration of oxygen, after the turning point has been reached, the respiration may again fail and the blood goes profoundly acid (pH circa 6.8). Even if the respiration does not stop completely, it is a matter of hours before the reaction of arterial blood again approaches normal values.

DISCUSSION. The changes in blood reaction observed are probably brought about by the increase in respiration and the consequent washing out of carbon dioxide, the blood consequently shifting in an alkaline direction. As the partial pressure of oxygen is lowered further a physiological limit is reached where the animal can no longer maintain respiration and as the respiration fails the blood then goes acid.

Because of these changes there are two precautions that must be observed in the use of oxygen at high altitudes. The first arises because of the alkalinity

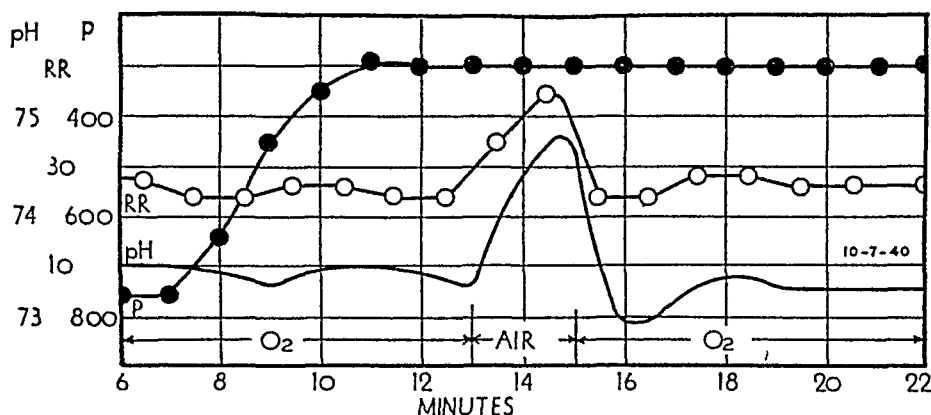


Fig. 3. Sudden anoxia. Dog, nembutal anesthesia. pH, —. Respiratory rate, R.R., — O —. Pressure, — ● —. Note the acid "overshoot" of the arterial blood.

and loss of CO_2 , for if oxygen is suddenly administered to an animal which has gone alkaline due to a loss of carbon dioxide, the animal may become apneic because of the lack of any adequate chemical stimulus for respiration. The second is the necessity of having a high enough oxygen pressure so that the turning point in blood reaction is not approached for if this point is reached the central respiratory mechanisms may be so severely damaged that they will fail to respond at all to adequate chemical stimuli.

These experiments suggest that if oxygen is used to combat the effects of lowered atmospheric pressure its administration should be started before the exposure to the lowered pressure. Moreover, if for any reason the oxygen supply is interrupted at high altitudes there is a serious danger not only during the period of anoxia but also after the oxygen supply is restored.

SUMMARY

1. Decompression in air is accompanied by an alkaline change in the blood and the change produced is roughly proportional to the reduction in pressure.

2. A physiological limit exists for each animal. This is characterized by a turning point in the relation of oxygen pressure to blood pH.

3. Administration of oxygen prevents a marked alkaline shift in blood.

4. The change in reaction of the blood is due to response of the respiratory mechanism to a reduced partial pressure of oxygen.

REFERENCE

NIMS, L. F. Yale J. Biol. Med. 10: 241, 1938.

THE RABBIT OVULATING FACTOR OF PLANT JUICE¹

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The presence of a substance in green plants which induces ovulation in rabbits, when injected intravenously, was demonstrated in a series of reports by Friedman and co-workers (1-4). The method of preparation and the method of testing the material suggested the possibility that, if the substance was a gonadotrophic agent, appropriate plant materials might be an economical source. The presence of this rabbit-ovulating factor in plant juice has been confirmed and the method of preparation has been simplified and improved (5). Storage of large quantities of the material as frozen juice, or as a dried precipitate, has made possible a study of the physiological characteristics of this material. The physiological evidence indicating that this ovulation factor is not gonadotrophic in nature is set forth in this communication.

MATERIALS AND METHODS. A number of green plant materials such as alfalfa, oats, corn, carrot tops and lawn grass cuttings have been shown to contain this rabbit ovulating factor; so there is no apparent restriction of the material to any taxonomic group. Plants were harvested and processed in the manner described by Borasky and Bradbury. The Oats 7 extracts used in the experiments listed here were made up from time to time from a powder prepared in May, 1942. This powder was prepared from a batch of juice that had been kept frozen from September, 1941.

During the first 2 years of this study the post-partum or the post-pseudopregnant rabbit was employed as the test animal. During this time it was shown that there is a seasonal variation in the sensitivity of the rabbit (5) and also that there is a high incidence of anestrus in post-partum rabbits during the winter months (6). Since these findings proved that the post-partum rabbit is not a uniformly responsive test animal, the later part of the study has been done using the less expensive, isolated or post-pseudopregnant estrous rabbit. The condition of estrus is judged by abdominal palpation of the uterus. The plant juice extracts are injected intravenously and the condition of the rabbit and its weight change are noted for 48 hours. At the time of laparotomy the appearance of the ovary and uterus is noted in confirmation of the diagnosis made by palpation. Ovaries in estrous rabbits are usually large but are chiefly characterized by being made up of an opaque white matrix. The anestrus or non-estrous ovary is small and transparent or translucent; it lacks the white

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opacity even though it may contain a number of vesicular follicles that appear normal. As a result of treatment, these non-estrous ovaries frequently form hemorrhagic and cystic hyperemic follicles which subsequently luteinize and the rabbit experiences a pseudopregnancy. Since these rabbits are not estrous and the follicles are not mature enough to ovulate, we have indicated this luteinization of unruptured follicles as a plus-minus reaction. For assay purposes, this plus-minus reaction is considered as a positive reaction in a rabbit that is not fully estrous.

Rabbit pituitaries are removed immediately after sacrificing the animal. The pituitaries are weighed to the nearest 0.5 mgm. on a Roller-Smith torsion balance and then placed between 2 microscopic slides. Using 1 slide as a spatula the pituitary is crushed and worked into a smooth paste between the slides and then the 2 slides are placed in a vial and dilute alkali is added in an amount equivalent to 1 cc. to each 5 mgm. of fresh pituitary substance. This dilute alkali (1 drop N/10 NaOH in 10 cc. distilled water) quickly extracts and dissolves the tissue from the glass slides and the solution is injected into test rabbits. Assay dosages are expressed as milligrams of fresh pituitary per kilogram of body weight and at least 3 rabbits are used at each dose level.

EXPERIMENTAL. These plant extracts may be so toxic that they can be tested only within a limited dose range. The nature of the toxicity is not known but there is commonly a marked alteration in the respiratory rhythm within 5 to 10 minutes. Within 30 minutes to an hour there may be an audible dyspnea due to an accumulation of mucus which may persist for 1 or 2 days. In the early part of this study we isolated a number of these rabbits for snuffles on the basis of the apparent rhinitis the day after the plant juice extracts had been injected. Injections of atropine were occasionally employed to relieve the respiratory difficulties due to the accumulation of mucus. Larger doses of plant juice extract may cause death immediately or within 10 to 20 minutes. In these cases there is a convulsive seizure and acute air hunger suggestive of an embolism. The rabbits take very little food or water for 24 to 48 hours after an injection of the plant extract and there is usually a marked loss in body weight within 48 hours. Diarrhea is also commonly present in this interval.

The data shown in table 1 are typical of the results obtained when groups of rabbits are injected with plant juice extracts at different seasons of the year. Weight loss may vary in degree but it is quite consistently present. Corn 2 was an extract of the initial acid precipitate and was relatively low in toxicity. The decreased incidence of positive results in September (Oats 7, table 1) is confirmation of the seasonal variation in the sensitivity of the test rabbits noted by Borasky and Bradbury. This seasonal effect was again confirmed in 1943 and probably accounts for some of the results of Friedman and Mitchell.

It has been found that if anestrous rabbits are given a priming treatment with estrogen they will ovulate when given an injection of copper acetate (7). A series of experiments was conducted to determine whether estrogen pretreatment would make anestrous rabbits more responsive to plant juice extracts. Six anestrous rabbits were given 8,000 I. U. of estrogen subcutaneously and the

following day were given an intravenous injection of Oats 7 extract in a dose of 2 mgm. per kilogram. Five of these rabbits responded, 2 ovulated and 3 had

TABLE 1
Response of rabbits injected with plant juice extracts

DOSE	BODY WEIGHT		OVULATION RESPONSE	REMARKS
	Initial	After 48 hr.		
Corn 2 (7/1/42)				
<i>mgm./kgm.</i>	<i>kgm.</i>	<i>kgm.</i>		
20	4.2	4.3	+	
10	3.3	3.1	+	
7/21/42 (Same solution)				
10	4.4	4.0	+	
5	3.2	3.0	+	
5	3.4	3.2	—	
Oats 7 (7/1/42)				
10	4.3	3.6	±	Died 7/6
4	3.5	3.2	+	
2	3.8	3.4	+	Died 7/6
1	3.7	3.7	+	
1	5.1	4.8	+	
1	4.3	4.0	—	Anestric
1	3.1	2.8	—	Anestric
0.5	2.7	2.6	—	
0.5	2.7	2.5	±	
Oats 7 (9/8/42)				
6.0	3.1			Died 6-8 hours
4.5	4.5		—	Died 12-20 hours
3.0	3.2			Died 4 hours
2.0	3.3	3.3	—	
2.0	3.1	3.1	—	
1.5	4.2	3.7	±	Died 48-96 hours
1.5	4.1	3.9	—	
1.0	3.2	3.2	—	
1.0	2.9	2.9	—	
Oats 7 (1/30/43)				
2.0	3.8	3.6	—	
2.0	3.6	3.3	+	
2.0	4.3	4.0	+	
2.0	3.9	3.5	+	
2.0	3.8	3.6	—	

follicles that luteinized without ovulation; the sixth died in about 12 hours. This same solution had not produced any positive results in 4 anestrus rabbits

injected 2 weeks previously. In a subsequent experiment 4 out of 7 estrogen primed rabbits ovulated when a dose of 1 mgm. per kilogram of oats was given, whereas, of the 6 unprimed rabbits that survived an injection of oat juice, 2 showed a plus-minus response and 4 were negative. The results of these experiments indicate that estrogen pretreatment increases the sensitivity of the anestrus rabbit to the plant juice factor. This increase in responsiveness of the estrogen primed anestrus rabbit to plant juice extract, and to copper acetate, is so similar that it suggests that the two substances act in the same manner.

The results of Brooks, Beadenkopf and Bojar (8) indicated that copper salts activated a nervous mechanism which caused a release of gonadotrophic hormone from the pituitary. There are no published data to show whether there is a demonstrable discharge of hormone from the pituitary following the injection of copper acetate so pituitaries from rabbits ovulated with plant juice extracts and also with copper were assayed. Pituitaries were taken from rabbits which had been injected with plant juice or copper acetate and the potency of those which had ovulated was compared with those which had not ovulated. Selection on this basis insured that all the donors had been subjected to the toxic effects of the extract for 48 hours previous to autopsy.

Two rabbits were injected with an extract of Oats 6 (2 mgm. per kgm.). In one the initial body weight was 5.6 kgm.; 48 hours later the weight was 5.0 kgm., 5 ruptured follicles were present and the ovarian weight was 0.98 gram. The other rabbit had an initial weight of 4.4 kgm.; after 48 hours the weight was 4.0 kgm., the ovaries were negative and weighed 1.06 grams. The pituitaries were extracted in dilute alkali, 5 mgm. fresh tissue per cubic centimeter for assay. The results of the assay show that the pituitary from the rabbit ovulated with Oats 6 had less hormone than that of the non-ovulated rabbit (table 2). In another test, 5 anestrus rabbits were pretreated with estrogen and then were injected with Oats 7 at a dose of 1.2 mgm. per kilogram. Two died within 4 hours, another after 36 to 48 hours and it had 2 ruptured follicles and the remaining two rabbits survived 48 hours. One had lost weight from 4.4 to 4.1 kgm.; its ovaries weighed 0.6 gram and were negative. The other rabbit lost weight, 4.8 to 4.3 kgm., its ovaries weighed 0.6 gram and contained 4 ruptured follicles. The pituitaries from these rabbits were assayed and the ovulated pituitary was found to be less potent (table 2).

A similar group of assays was made on pituitaries from rabbits which had been treated with copper acetate. As an example, 6 anestrus rabbits were pretreated with estrogen and then injected with copper in the manner described previously (7). The dose of copper acetate was 2.5 mgm. per kilogram, which is tolerated without any untoward symptoms or weight loss. Three of these rabbits ovulated and their pituitaries were pooled and assayed. The other 3 failed to ovulate and their pituitaries were likewise pooled and assayed. The results of the comparative assay showed that the pituitaries from the non-ovulated rabbits were about twice as potent as the pituitaries from the ovulated rabbits (table 2). Similar assay results were obtained in two experiments in which ovulation was induced in estrous rabbits by the injection of copper. In other assays it has

been found that the potency of the pituitary from treated non-ovulated rabbit is entirely comparable to that of an untreated control rabbit. Thus ovulation occurs after copper or plant juice injections, only when there is an appreciable discharge of hormone from the pituitary.

These assay results are interpreted as showing that the rabbit-ovulating factor in plant juice has an action very similar to that of copper acetate in that both substances cause a release of hormone from the rabbit pituitary in a quantity which is more than adequate to induce the ovulation observed after treatment.

TABLE 2

Assay of pituitaries from rabbits injected with plant juice extract

PITUITARIES	DOSE OF PITUITARY	NUMBER OF TEST RABBITS RESPONDING		
		—	±	+
Oats 6 (4/10/43)				
Ovulated	<i>mgm./kgm.</i> 0.50	3	1	
	0.70	3		1
Non-ovulated	0.50	1		3
	0.70		2	2
Oats 7 (2/14/44)				
Ovulated	0.75	6		
	1.0			2
Non-ovulated	0.75		2	2
	1.0		1	
Copper acetate (1/19/44)				
Ovulated	2.0			3
	1.5	1	1	1
	1.0	3		
Non-ovulated	1.0			3
	0.75	1	1	1
	0.50	3		

The rabbit ovulation factors from plant juice and copper acetate are also similar in that they are effective only in estrous or estrogen primed rabbits.

A number of substances such as copper and heme, which have no gonadotrophic effect in the immature rat when given alone, have been used to augment the effect of pituitary gonadotrophic extracts in the immature rat (9, 10). A series of experiments was done in which the active preparations of O6 and O7 were injected into immature male and female rats alone and together with a pituitary F.S.H. preparation.

A solution of Oats 6 containing 8.9 mgm. per cubic centimeter was injected

into a group of immature female rats. Six injections of 0.2 cc. each made a total dose of 10.6 mgm. per rat. (Two milligrams per kilogram of this solution had ovulated a rabbit, table 2.) The results of this experiment when Oats 6 was given alone, or together with F.S.H., are listed in table 3. There is no appreciable effect of Oats 6, except when mixed in vitro and injected with F.S.H. it may have delayed the absorption of F.S.H. enough to make it more effective. However when Oats 6 and F.S.H. were injected at separate sites (one dorsal and the other ventral) it had no effect. Similar results were obtained when a solution of Oats 7 was used (1.2 mgm. per kilogram of this solution ovulated a rabbit). The total dose was 7.5 mgm. and again the only apparent effect may have been due to a delayed absorption of the F.S.H. (table 3). Mixing the Oats extracts with the F.S.H. solution before injection doubled the quantity of fluid at the injection site, which may also have been a factor in the rate of absorption but the increased effect was not of the order of magnitude which is recognized as

TABLE 3
Ineffectiveness of oat juice extracts in the immature female rat

	NO. OF RATS	AV. OVARIAN WEIGHT	AV. UTERINE WEIGHT
Oats 6.....	3	17.3	30.0
Oats 6 + F.S.H. mixed in vitro.....	3	31.6	92.0
Oats 6 + F.S.H. injected separately.....	3	26.0	92.0
F.S.H.....	5	25.2	92.0
Oats 7.....	3	15.3	27.0
Oats 7 + F.S.H. mixed in vitro.....	3	31.8	69.0
Oats 7 + F.S.H. injected separately.....	3	24.1	69.0
F.S.H.	3	23.1	73.0

Untreated control ovaries range from 12 to 20 mgm. with group averages commonly 14 to 16 mgm.; oats 6, total dose 10.6 mgm.; oats 7, total dose 7.5 mgm.; F.S.H., total dose 0.14 mgm.

augmentation. The results with immature males were also negative for any demonstrable gonadotrophic or augmenting effects of these oat juice extracts. The lack of any effect when given alone, and the lack of any augmentation when given together with F.S.H. indicates that the plant juice substance does not possess the attributes of either of the pituitary gonadotrophins, F.S.H. or L.H.

DISCUSSION. Peysakhovich (11) reported that an extract of the red onion produced gonadotrophic effects in rabbits and in immature rats. His notations on the technique of preparation are so incomplete and contradictory that it was not possible to confirm his report. He stated that his material was destroyed by boiling in aqueous solution. The substance in our extracts is heat stable since we have obtained it from boiled plant juice, and the extracts have retained their potency after boiling. In this respect the ovulating factor is more like the yeast extracts of Fevold, Hisaw and Greep in which the active agent was heat stable and was assumed to be copper. However, unless copper is present in some

organic combination which is more effective than copper acetate, the doses of our plant juice extracts are too small for copper to be the effective agent.

It was found that an intravenous dose of picrotoxin, which was lethal in 20 per cent of the cases and convulsive in 75 per cent, often caused ovulation or ovarian stimulation in rabbits (12). Emmens (13) concluded that copper and cadmium produced ovulation in the same manner as picrotoxin: by causing a release of hormone from the pituitary. The experimental evidence therefore seems to indicate that the rabbit ovulating factor of plant juice may be effective by virtue of its toxicity. However, should chemical purification reveal that the toxic substance can be separated from the ovulating principle, the fact that it acts by causing a discharge of hormone from the pituitary suggests that it is a pituitary secretagogue rather than a gonadotrophic agent. The physiological demonstration of this secretagogue effect will probably be limited to the rabbit, cat and ferret; species in which ovulation normally results from copulatory stimulation.

SUMMARY

1. Estrogen pretreatment increases the sensitivity of the anestrus rabbit to the ovulation effects of plant juice extracts.

2. The rabbit ovulating factor of plant juice is an agent that causes a release of hormone from the pituitary of the estrus or estrogen primed rabbit in quantities sufficient to cause the ovulation.

3. Plant juice extracts which were capable of causing ovulation in rabbits were ineffective in infantile rats either when given alone or in combination with pituitary extracts.

4. The physiological evidence indicates that the factor in plant juice may exert its effect as a neurotoxic agent and that it does not have the attributes of a gonadotrophic substance.

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REFERENCES

- (1) FRIEDMAN, M. H. AND G. S. FRIEDMAN. *Proc. Soc. Exper. Biol. and Med.* **31**: 842, 1934.
- (2) FRIEDMAN, M. H. *Proc. Soc. Exper. Biol. and Med.* **37**: 645, 1938.
- (3) FRIEDMAN, M. H. AND G. S. FRIEDMAN. *This Journal* **125**: 486, 1939.
- (4) FRIEDMAN, M. H. AND J. W. MITCHELL. *Endocrinology* **29**: 172, 1941.
- (5) BORASKY, R. AND J. T. BRADBURY. *This Journal* **137**: 637, 1942.
- (6) BRADBURY, J. T. *Anat. Record* **88**: 424, 1944.
- (7) DURY, A. AND J. T. BRADBURY. *This Journal* **139**: 135, 1943.
- (8) BROOKS, C. M., W. G. BEADENKOPF AND S. BOJAR. *Endocrinology* **27**: 878, 1940.
- (9) FEVOLD, H. L., F. L. HISAW AND R. O. GREEP. *This Journal* **117**: 68, 1936.
- (10) McSHAN, W. H. AND R. K. MEYER. *Endocrinology* **28**: 694, 1941.
- (11) PEYSAKHOVICH, I. M. *Vrachebnoe delo* **16**: 775, 1933.
- (12) MARSHALL, F. H. A., E. B. VERNEY AND M. VOGT. *J. Physiol.* **97**: 128, 1939.
- (13) EMMENS, C. W. *J. Endocrinology* **2**: 63, 1940.

ISCHEMIC COMPRESSION SHOCK, WITH AN ANALYSIS OF LOCAL FLUID LOSS¹

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Crushing injuries may lead either to a delayed renal disturbance known as crush syndrome (1, 2) or to an immediate state of progressive circulatory failure which belongs to the category of traumatic shock. This report deals with the shock state accompanying an ischemic form of compression of the hind legs of anesthetized dogs in which local fluid loss and hemorrhage were minimal.

METHODS. Dogs, weighing 10 to 20 kgm., and anesthetized with an initial subcutaneous injection of approximately 4 mgm./kgm. of morphine plus an intravenous injection of 20 mgm./kgm. of sodium pentobarbital or an initial injection of 25 to 30 mgm./kgm. of sodium pentobarbital alone, were traumatized by wrapping each hind leg from the ankle to groin with a continuous spiral of 2 to 3 m. of rubber tubing of approximately 14 mm. outside diameter and 7 to 9 mm. inside diameter pulled to 10 to 15 kgm. tension. While the tissue tension was not measured it is computed that in the thigh it amounted to about 4 kgm./cm.² This method is a partial adaptation of the procedure used by Eggleton, Richardson, Schild and Winton (1) to study the crush syndrome.

In one group of experiments the dogs were placed in recovery cages immediately after wrapping the tubes about the legs. Mean arterial pressure was recorded with the Hg manometer from a carotid artery of a second group of dogs. Hematocrit readings were made of the arterial blood of these dogs and by venipuncture in the first group. Wintrobe hematocrit tubes were used and were spun for 30 min. at 2,000 R.P.M. Tubes spun a second time for 30 min. at 3000 R.P.M. on another centrifuge decreased in reading by only 1 cell volume per cent. Rectal, subcutaneous and intramuscular temperatures were recorded (3).

All dogs had been in the kennels at least one week and were in good condition when used for the experiments. Food was withheld for 20 hrs. preceding the experiment. Water was available up to the time of anesthetization, but no further water was given for 30 hrs.

RESULTS. *A. Duration of compression and survival.* The duration of compression and the survival of all dogs used in this study are indicated in table 1, A-D. The survival of 14 control dogs is indicated in table 2.

B. Reactions after release of the rubber tubes. A few of the dogs placed in cages were given small additional injections of morphine during the period of compression, but most of them did not require further anesthesia. Almost all were attempting to stand when the rubber tubes were removed, but within a few minutes they all became stuporous and many remained so until death. Those that survived many hours subsequently became partially awake and attempted to sit up, but they again became semicomatose with rapid shallow respirations, characterized by the presence of forced expirations, shortly before death. Five of the dogs whose mean arterial pressure was recorded required additional small

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TABLE 1
Ischemic compression shock

TYPE OF EXP.	NO. OF DOGS	COMPRES- SION—HRS.			SURVIVAL AFTER RELEASE OF COMPRESSION— HRS.			EDEMA ML./KGM.								AUTOPSY OF LEGS—NO. OF DOGS IN EACH CATEGORY ^b						ESTIMATED PLASMA LOSS ^c			
								Measured edema		Est. Max. edema ^a												I		II	
		Range	Av.	Median	Range	Av.	σ	No. of dogs	Range	Av.	Range	Av.	σ	$\Sigma\sigma$	0	+	++	+++	++++	Range	Av.	Range	Av.		
A	4	3-6	4	3.5	Survived	0.8-31	8.6	9	0																
	15	5-20.8	8	6					4	3.5-35	17	15	-49	30	14	8.5	1	7	2	1	0				
B	9	6-6.3	6.1	6.1	4-50	16	14.1	9	26-41	36	39	-56	46	6.5	6	0	0	5	1	3	12	-27	22	9-32	21
C	14	6-16.5	7.6	6.7	2-20	7.9	5.5	13	-7-20	6	3.3-33	18	8	7	7	4	0	2	0		6.5-29	18	1-26	11	
D	4 ^d	6-6.3	6.1	6.1	18-24	20.6		4	13-37	24	20	-47	32		3	0	0	0	4	0	11	-27	19	14-24	19
	3 ^e	6-6.1	6.1	6.1	9.3-13	11.1		3	20-45	30	33	-55	39	2.5	0	0	0	1	2	19	-25	22	18-18	18	

A—both hind legs traumatized, dogs minimally anesthetized, free to move about in cages, studied during December to February.

B—the same as A but studied in May.

C—both hind legs traumatized, kept on animal boards and mean arterial pressure recorded continuously, studied during December to February.

D—one hind leg traumatized, minimally anesthetized, free to move about in cages, studied during May.

Av.—average.

σ —standard deviation of individual determinations about the average, computed as:

$$\sigma = \sqrt{\frac{\sum(\bar{X} - X)^2}{N - 1}}$$

where \bar{X} = average of determinations on all animals in the group, X = individual determination on each animal and N = number of animals.

a = (measured edema + 10)1.10—see p. 501 for explanation.

$\Sigma\sigma$ —This is an expression for the probable error of the estimated maximum edema. It was computed as follows: For each measurement of limb volume several immersions were made. The standard deviation of the difference in volume for an extremity was computed as:

$$\sigma_I = \sqrt{\frac{\sum[\bar{X}_c - X_c]^2 + \sum[\bar{X}_{pm} - X_{pm}]^2}{N_c - 1 + N_{pm} - 1}}$$

where X_c is the individual measurements at each immersion and \bar{X}_c is the mean of these measurements during the control period, and X_{pm} and \bar{X}_{pm} are the corresponding measurements after death; and where N_c and N_{pm} are the number of immersions respectively. Letting σ_b be the sum of σ_I for the two legs, and using σ_s , the standard deviation for the change in volume of a leg with death, = 2.0 [table 4], then the total standard deviation for the estimated maximum edema in a dog is:

$$\Sigma\sigma = \sigma_b + 2 \times \sigma_s.$$

In the above table the column labelled $\Sigma\sigma$ is the average of the $\Sigma\sigma$ for all of the dogs in the group.

b—see p. 498 for explanation.

c—plasma loss was roughly computed as = $80 \left[1 - \frac{H_c}{H_p} \right]$, where 80 = the estimated blood volume in ml./kgm., and H_c and H_p are the control and postmortem hematocrit readings respectively.

I—control hematocrit taken just before anesthetization.

II—control hematocrit taken just before release of rubber tubes on hind legs.

d—killed; e—died.

injections of sodium pentobarbital during the period of compression. Only one of these required a small additional amount of anesthetic after release of the tubes.

In the dogs listed in table 1 C, the mean arterial pressure remained around 170

TABLE 2
Controls—No trauma

	NO. OF DOGS	ON ANIMAL BOARDS FOR—HRS.		DIED IN—HRS.	
		Range	Av.	Range	Av.
On animal boards, mean arterial pressure recorded for indicated number of hours, dogs then placed in cages	2	8-25	16.5	8-28.5	18
	6	10-25	15	Survived	
In cages continuously	1		0	24	24
	5		0	Survived	

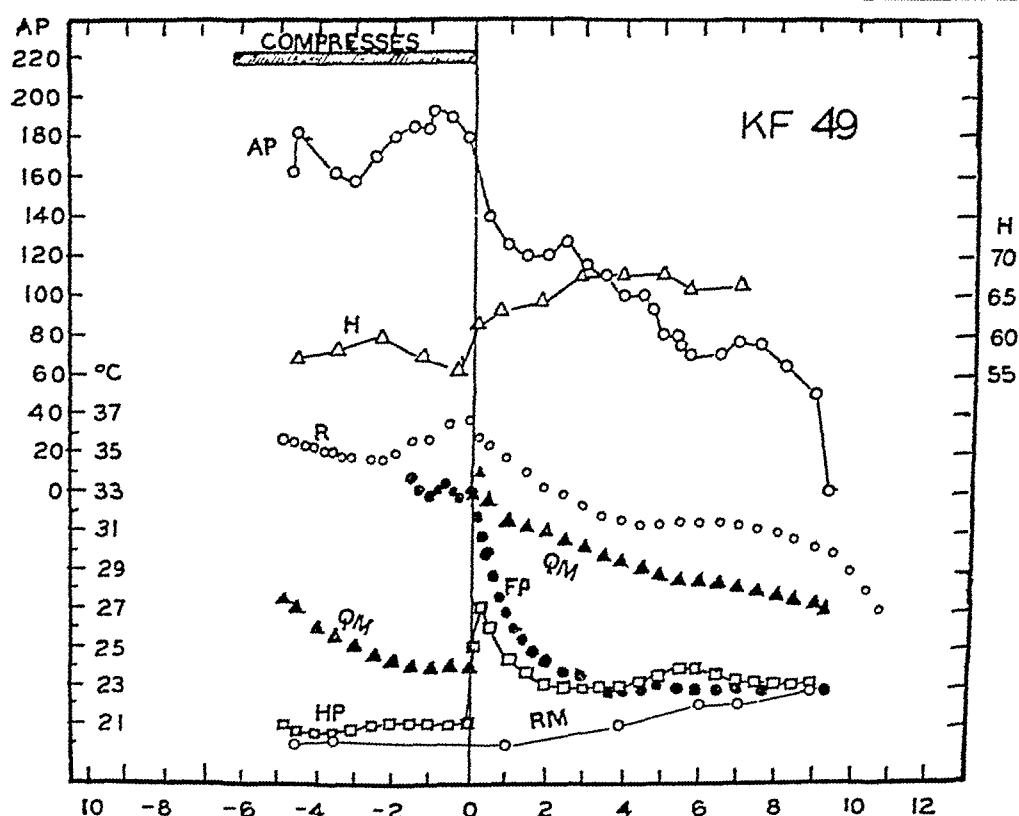


Fig. 1. Consecutive changes in mean arterial pressure, etc., in a dog subjected to ischemic compression of its hind legs. Anesthesia 25 mgm./kgm. of sodium pentobarbital at minus 7.25 hours. Duration of compression of hind extremities indicated by shaded bar at top. Zero time—release of rubber tubes. AP—mean arterial pressure, H—hematocrit reading, R—rectal temperature, FP—subcutaneous temperature of forepaws, QM—temperature of quadriceps muscle, HP—subcutaneous temperature of hindpaw, RM—room temperature. Abscissal scale—time in hours. Ordinate scales: AP—mean arterial pressure in millimeter Hg, H—hematocrit reading in cell volume per cent of blood volume, °C—scale for temperature readings in centigrade.

to 180 mm.Hg during the latter part of the period of limb compression. It fell rapidly during the first 15 minutes to 1 hour after release of compression to 120 to 150 mm.Hg and then more gradually. When the mean arterial pressure reached 50 to 60 mm.Hg the circulation or respiration usually rapidly failed and

the animal died within the ensuing 20 to 30 minutes. The results in a typical experiment are illustrated in figure 1. Venous pressure was not recorded, but it was observed that after release of the rubber tubes venapuncture in the fore leg was much more difficult because of the failure of the vein to fill, and the withdrawn blood was much darker. Because of the rapid heart rates in dogs anesthetized with pentobarbital, three experiments were performed under morphine and evipal. In two of these the heart rate ranged between 50 and 70 and in the third between 85 and 135 beats per minute during the period of limb compression. In each the heart rate rose rapidly immediately after removal of the rubber tubes, exceeding 160 within 15 minutes and reaching a maximum of 240 to 260 beats per minute within 2 hours or less. The mean arterial pressure did not drop below 90 to 100 mm.Hg until the heart rate had reached its maximum.

The plots in figure 2 show that in almost all animals the hematocrit reading rose 5 to 10 cell volumes per cent shortly after release of the rubber tubes and

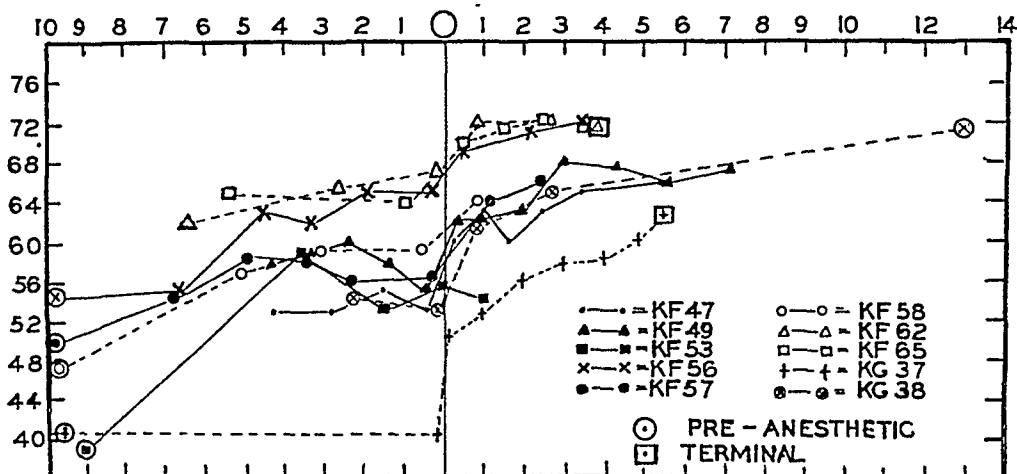


Fig. 2. Consecutive changes in hematocrit readings in all experiments in which studied. Scales—same as figure 1.

continued to rise more slowly thereafter. In several of the experiments the hematocrit reading rose considerably during the period of compression. This rise may have been an effect of the anesthetic (3) or possibly in some way dependent upon the limb compression. No satisfactory correlation was found between the magnitude of the rise in hematocrit reading and either the volume of edema or the survival.

The changes in subcutaneous and intramuscular temperature in the traumatized extremities were similar in all experiments. Those in a typical experiment are illustrated in figure 1. As soon as the tubes were applied, these temperatures began to fall, and usually were approximately at room temperature when the tubes were released. Immediately upon release of the rubber tubes both temperatures rapidly rose, but within 15 to 30 minutes they again began to decline. The subcutaneous temperature rapidly approached room temperature, while the intramuscular temperature returned to a position midway between rectal and room temperature.

The subcutaneous temperature of the untraumatized forepaws, recorded in three experiments during the winter months, was within 2 to 5° of the rectal temperature during the compression period. Within a few minutes after release of the rubber tubes the subcutaneous temperature began to drop and rapidly approached room temperature. In view of studies reported previously (3, 4) it is apparent that the blood flow through the skin of the forepaw was rather promptly reduced after release of compression of the hindlegs and remained at a very low rate despite the fact that initially the mean arterial pressure was still quite high. These changes are illustrated in figure 1. In this experiment the rectal temperature declined during the period of falling arterial pressure. More commonly, the rectal temperature remained steady or rose progressively until death.

Except for those which survived more than 24 hours, none of the dogs voided after release of the rubber tubes. The urine, found in the bladder at autopsy in quantities of 5 to 300 ml., was frequently deeply pigmented, indicating a concentrated urine, but only a few specimens had a reddish tinge. Five of the latter were examined spectroscopically for us by Dr. Wade Price of the Department of Medicine. He found evidence of myoglobin in 2, KG-55 and KG-56, a trace of myoglobin in 1, KG-36, and no myoglobin in 2, KG-26 and KG-37. No hemoglobin was found in any of the specimens.

A few of the dogs were carefully examined several hours after release of the rubber tubes. Moderate power had returned to the proximal thigh muscles and a knee-jerk could be obtained. Muscles of the distal part of the leg could not be caused to contract reflexly, but could be excited by electrical stimuli applied by surface electrodes, and the animals seemed to be aware of the movement. The animals which survived in this as well as in another series of experiments (39) were able to use the hindlegs for balance, but for many days were unable to support the body with them. For as long as they were followed, the dogs walked on the dorsum of the hind feet. Superficial cutaneous ulcers developed in one dog, but no evidence of deep infection was observed.

C. Postmortem examinations. Gross postmortem examinations were performed on all dogs as soon as possible after death. These findings were controlled by many examinations of dogs rapidly bled to death or killed by ventricular fibrillation. In the traumatized legs (see table 1) the findings varied from normal—labeled 0 in the tables, just detectable moistness of the soft tissues—labeled +, prominent edema of the soft tissues—labeled ++, marked gelatinous edema of the soft tissues and moistness of the muscles—labeled +++, to very marked gelatinous edema of the soft tissues associated with marked edema and pallor of the muscles—labeled +++++. Occasionally areas of hemorrhage were seen in the muscles or soft tissues, but they were never very prominent. In the dogs dying in shock the tissues throughout the body varied from the *euhemic*³ appearance seen in dogs killed by ventricular fibrillation to a moderate

³ The word *euhemic* is proposed as descriptive of tissues having a normal blood content in contrast to the anemic or hyperemic appearance seen in abnormal states of the circulation.

hyperemia. The tissues differed markedly from the anemic appearance seen in dogs bled to death.

Subendocardial hemorrhages were seen in the atrial surface of the mitral valve in 22 dogs and in the tricuspid valve in 2. However, such subendocardial hemorrhages have been observed not infrequently in nontraumatized controls. In most of the dogs fine blood vessels were seen in the subcutaneous tissue over the thorax and in 10 not autopsied until 10 or more hours after death the subcutaneous fat was diffusely purple. The omentum, mesentery and serosa of the gut varied from euhemia to a marked hyperemia. The latter was particularly prominent in the dogs studied in May (39). A large number showed small hyperemic areas in the duodenal mucosa, but an identical appearance was seen in many of the control dogs. As with "hemorrhagic" and certain other forms of shock (5-10, 18), 7 dogs showed free blood in the lumen of the duodenum. In several dogs there were spotty, purple, hyperemic areas in the lungs. This finding was also particularly prominent in the dogs studied during May.

Complete microscopical studies were performed on two dogs which were observed during May. These studies⁴ revealed extensive passive hyperemia involving particularly the capillaries and veins. This hyperemia was seen in the myocardium, lungs, liver (particularly in the middle and central portions of the lobules), spleen and kidneys (associated with cloudy swelling of the convoluted tubules). The muscle of the traumatized legs showed what appeared to be rupture of the sarcolemma with retraction and tortuosity of the muscle fiber and edema in and between the muscle fibers but no hemorrhage. Inflammatory disease of the cardiac valves, myocardium, kidney and adrenal was found in both dogs but no inflammation was seen in the traumatized legs of either dog.

DISCUSSION. In a recent paper, Eggleton, Richardson, Schild and Winton (1) report that pounding the muscles of the hind legs plus application of rubber tubes in a tightly wound spiral from ankle to groin for 5 hours, plus crushing the compressed extremities in a vice for 5 minutes resulted in a decline of arterial pressure and a failure of renal function after release of the rubber tubes. They give, however, no data on survival or on the edema in the legs. We have adopted their method of applying the rubber tubes, but have found that the additional trauma is not necessary for the induction of a standard degree of shock in our dogs.

Production of shock by pounding an extremity was initiated by Cannon and Bayliss (11-13) in 1919. Since then many similar methods have been devised (14-27). The shock produced by these methods is due in part to the hemorrhage into the traumatized tissues and perhaps also to infection. Application of tourniquets composed of 2-3 turns of heavy rubber tubing wrapped about one or both thighs as close to the groin as possible has also been used by many investigators (28-37). The shock induced by this method is probably dependent upon the ischemia of the legs. Our method combines the effects of mechanical trauma with ischemia without complicating the experiment by causing any serious

⁴ These studies were made for us by Dr. Howard T. Karsner, director of the Department of Pathology.

amount of local hemorrhage and with a minimum opportunity for introduction of bacteria.

The behavior of the dogs used in this study during the hours following release of the tubes is similar to that seen in clinical shock. Thus, there is a depression of cerebral motor activity, an apparent cessation of the need for further anesthesia, collapse of the peripheral veins, hemoconcentration, and a progressive slow decline in arterial pressure ending in death.

The initial rapid rise in hematocrit reading is probably in part due to the formation of the edema in the legs, but it is also in part due to the cutaneous vasoconstriction and probably to retraction of the spleen (38). These latter possibilities plus a variable absorption of tissue fluid may account for the poor correlation between the volume of edema and the magnitude of the change of hematocrit.

The postmortem examinations indicate that the lungs, liver, fatty tissue of the abdomen, mesentery, omentum, thoracic structures and subcutaneous tissue, and the kidneys may all be the site of accumulation of blood, particularly in the capillaries and veins, which may be greater than that seen in animals dying suddenly of ventricular fibrillation or hemorrhage. It is not possible to state, however, whether this accumulation of blood played an active part in the final circulatory failure or whether the apparent hyperemia is due to the presence of a normal quantity of concentrated blood in the systemic and pulmonary venous systems after death. In view of the elevated hematocrit in these dogs the latter might very well explain the findings.

Local fluid accumulation. 1. Method. The local edema in each hindleg was computed from the difference between the volume of the extremity after death and that before traumatization. This volume, which included all proximal soft tissues of the thigh and groin up to the midline ventrally and, on the lateral and dorsal aspect of the thigh, to within 4 to 7 cm. of the midline in the cephalad direction and to the base of the tail caudally, was obtained by immersing the extremity in a tall narrow mouthed vessel, filled with a dilute solution of lysol in water, until the ischial tuberosity was depressed into, and the iliac crest, symphysis pubis and base of the tail rested upon the mouth of the vessel. During the immersion the torso lay upon a platform the same height as the immersion vessel and the opposite extremity was extended and elevated approximately 40° above the horizontal. The depth to which the skin entered the water, marked with quick drying paint, was 6 to 10 cm. above the highest level of compression on the lateral aspect of the thigh. At least 2 and usually 3 or 4 immersions were made at each determination of volume.

One possible error in this method of measuring the edema is that the legs might shrink at the time of death due to loss of turgor and perhaps also to vasoconstriction in the vascular beds of the extremities. As a result, the apparent edema would be less than the actual edema by the amount of such shrinkage. This was tested by immersing an extremity before and again after death due to various causes. Table 3 shows that in most animals the shrinkage was approximately 3 ml./kgm. of body weight per extremity, whereas in 7 edematous extremities, the shrinkage was approximately 5 ml./kgm. of body weight per extremity. Evidently we shall have to add the latter amount to the measured edema to obtain the true volume of edema.

Despite the fact that the legs were immersed to a level well above the highest region of compression it is still possible that some edema in proximal soft tissues or in the pelvis was missed. This possibility was tested in several experiments by applying a rubber tube to only one extremity. The tube was removed in 6 hours and, after the dog had died or had been killed, the edema of the traumatized extremity, estimated by immersion, was compared

with the difference in weight between the control and traumatized extremities, severed according to the method Blalock (14, 16) except that the sacrum was sawed in two instead of being disarticulated. The results are presented in table 4. Column D shows that the measured volume gain ranged from 63 to 88 per cent of the difference in weight between the two extremities. Since we consider that an edematous leg may shrink 5 ml./kgm. of body weight we have added 5 ml. to the measured volume. We have also multiplied this sum by 1.03 to allow for the density of the edema fluid. The resulting figure is tabulated in column E. Column F shows that when these two corrections are made, the resulting figure is 90 to 107

TABLE 3

Modification in the volume of a leg resulting from death or compression

	NO. OF EXP.	MODIFICATION OF THE VOLUME IN ML./KGM.			
		Range	Average	Median	σ
A. Untraumatized legs*.....	19	-6.6 to +1.0	-3.3	-3.7	2.12
B. Traumatized legs†.....	7	-7.9 to -2.6	-4.8	-4.7	2.00
C. Compressed legs‡.....	14	-7.2 to -0.7	-4.3	-4.1	2.25

* Change in volume of normal legs as a result of accidental death or of death by bleeding or by ventricular fibrillation. No significant difference was found either between the various modes of death or between the legs whose opposite leg was edematous as a result of trauma and those whose opposite leg was normal.

† Legs were traumatized by ischemic compression, and after considerable edema had developed the limb volume was measured by immersion, the dogs were killed and the limb volume again measured.

‡ Limb volumes were measured, limbs were compressed for 6 hours, dogs were killed before removal of compression and after removal of the tubes the limb volumes were again measured.

TABLE 4

Comparison of dissection with immersion—1 leg traumatized

NO.	EXP. NO.	A DIFF. IN WT. OF LEGS BY DISSECTION	B DIFF. IN VOL. OF TRAUMA- TIZED LEG	C DIFF. IN VOL. OF CONTROL LEG	D B/A \times 100	E [B + 5] \times 1.03	F E/A \times 100
		gm./kgm.	ml /kgm.	ml./kgm.			
1	KG 44	34.6	25.4	-3.6	74	31.3	91
2	KG 45	32.1	24.2	-2.8	76	30.1	94
3	KG 47	20.2	12.7	-4.3	63	18.2	90
4	KG 48	26.5	20.3	-4.3	77	26.0	98
5	KG 49	28.6	20.8	-2.2	73	26.6	93
6	KG 62	38.5	35.0	-4.8	88	41.2	107
7	KG 65	57.7	45.3	-3.8	79	51.7	90

per cent of the difference in weight between the traumatized and control extremities. Since in most of these experiments the percentage is greater than 91, we believe that it is a safe assumption that only 10 per cent ($9/91 \times 100$) needs to be added to account for unmeasured proximal edema.

2. *Results.* The measured volume of edema and the estimated maximum volume of edema, allowing for the above corrections, are given in table 1, A-D for all

experiments in which they were measured. For comparison with these we have placed in table 5 the volumes of blood, expressed in milliliters per kilogram of body weight which had to be removed from similarly anesthetized normal dogs, in order either to bleed them to death (A, B) or in order to lower their mean arterial pressure to, and to keep it at 30 to 70 mm.Hg for 1.9 to 7.0 hours (C).

DISCUSSION. The initial decline in arterial pressure in these dogs is certainly due in part to a decreased total peripheral resistance caused by the reactive hyperemia in the traumatized legs as shown by the immediate elevation in their temperature. However, at most this can have caused only a temporary decline in arterial pressure since the temperature studies indicate that blood flow in the traumatized extremities returned to below normal within approximately an hour.

The progressive fall in arterial pressure up to death is probably dependent upon a continuing decrease in cardiac output dependent principally upon a gradually increasing discrepancy between vascular capacity and circulating blood vol-

TABLE 5
Bleeding volumes

	NO. OF EXP.	DURATION OF BLEEDING—HRS.		VOLUME REMOVED—ML./KGM.				HYPOTENSIVE PERIOD			
		Range	Average	Range	Average	Median	s	Duration—Hrs.		Mean Art P.—mm. Hg	
								Range	Ave.	Range	Av.
A. Normal anesthetized dogs.	4	0.3-2.5	1.6	48.0-69.0	57.0	57.5	8.66	Bled to death			
B. Bled before rubber tubes removed.	7	0.3-1.0	0.5	39.4-60.0	50.7	50.8	6.81	Bled to death			
C. Hemorrhagic hypotensive shock*.....	9	0.8-6.5	2.1	40.0-58.2	48.6	48.8	7.41	1.9-7.0	2.6	30-70	44.4

* Blood pressure recorded continuously. The survival time ranged from 0.3 to 8.0 hours with an average of 3.8 hours after reinfusion.

ume (38). The rather immediate depression of the nervous system and the abrupt decline in pressure suggest that nervous mechanisms or the activity of humoral substances may have contributed to the circulatory failure. Regarding these possibilities, this paper provides no direct evidence.

The cats studied by Cannon and Bayliss (11-13) died with a gain in weight of the traumatized extremity of less than 11 per cent of the estimated blood volume. They concluded on the basis of this and other observations that "the shock was due to the absorption of a depressant substance liberated by the traumatized tissues." Since they had severed the traumatized and untraumatized thighs by disarticulation at the knees and hips and had skinned the extremities before weighing they may not have measured all the edema. Most recent investigators, following the lead of Blalock (14, 16), have severed the control and traumatized legs, after making a trans-abdominal incision, by disarticulating the sacrum from the tail and sacral vertebrae. We have adopted the immersion method of

measuring the edema since it allows us to traumatize both extremities and thus obtain shock more consistently, is quicker, and makes it possible to measure the edema of surviving dogs (39). With the corrections discussed under methods, it appears to give a satisfactory measure of the local fluid loss.

The edema in the dogs in table 1A, and particularly those studied in May (table 1B), was in general rather large, as large in fact as that which most other investigators have found (table 6) in dogs dying of traumatic shock. In these experiments it is possible that the edema was the principal factor leading to the circulatory failure. It should be noted, however, that contrary to the technique used by most investigators, our dogs were minimally anesthetized and were free to move about in their cages. The experiments in table 1C, in which the dogs

TABLE 6
Summary of data in literature
Dogs which died as a result of the trauma

AUTHOR (REF.)	NO. OF DOGS USED	ANESTHESIA	TRAUMA	SURVIVAL TIME		EDEMA		
				Range	Av.	Range	Average	Method used
				<i>hrs.</i>	<i>hrs.</i>	<i>ml./kgm.</i>	<i>ml./kgm.</i>	
Parson and Phemister (15)	6*	Ether Morphine Na barbital	Pounding			33 -47	42.8	Dissection
Blalock (16).....	5*	Na barbital	Mild pounding	19 -40	24	24.6-48.1	36.0	Dissection
Wilson and Roome (31).....	13†	Na barbital	Rubber tourniquet	5 -52	25.6	10 -61	35.4	Dissection
Katz et al. (45).....	13*		Occlusion of venous return	5.3-13.5	7.4	35 -69	52.0	Dissection
Manery and Solandt (25)...	24†	Ether	Pounding	1.2-24	4.1	0 -57	32	Immersion
Green et al. (44).....	5*	Morphine Na barbital	Deep muscle burn	19 -35	25.7	0 -18	8.5	Immersion

* Mean arterial pressure recorded.

† Arterial pressure not recorded, but dogs apparently kept on animal boards.

were kept restricted to animal boards and in which the anesthetic was more prolonged, are more nearly comparable to those reported in the literature. In these the estimated maximum edema was quite small (3.3 to 33, average 18 ml./kgm. of body weight).

One possible explanation for the small volume of local fluid loss in the latter experiments is that we failed to measure all the edema. This does not seem likely since in all cases we have allowed the maximum error in computing the estimated maximum edema. Furthermore, the gross examination of the traumatized legs checks well with the immersion measurements.

A small amount of sticky fluid is often found adherent to the rubber tubes when they are removed. This fluid is in part water which remained on the legs after

the immersion, but it seems to be in part also a plasma-like substance squeezed through the skin by the compression of the rubber tubes. If a significant volume of tissue fluid were lost from the legs while they were being compressed then the replacement of this fluid upon release of the rubber tubes would cause a loss of body fluid which would not be indicated by the immersion measurements. To measure the magnitude of this possible fluid loss we wrapped the legs of several dogs and then killed them after 6 hours of compression, but before removing the tubes. Table 3C shows that the volume of the legs after 6 hours of compression is approximately 4.3 ml./kgm. of body weight less than that before wrapping. Since this is but little more than the amount by which untraumatized legs shrink with death (table 3A), we believe that the slightly greater decrease in volume is due primarily to a more complete squeezing of blood from the vessels of the legs into the systemic circulation and that an insignificant volume of fluid is lost from the body as a direct result of the compression. However, even if the difference between the two shrinkage volumes, 2.0 ml./kgm. (1.0×2), were added to the estimated maximum edema, the total fluid loss would still be small for the dogs whose blood pressure was recorded continuously.

In practically all bleeding experiments (43, 46-49, and table 5) it is necessary to remove a volume of blood equal to or greater than 50 ml./kgm. of body weight in order to cause immediate death. Since in most of our dogs the fluid volumes lost were less than this, it is apparent that the circulatory failure is not due simply to the immediate effects of loss of circulating blood volume. Loss of a smaller blood volume may, however, reduce the minute volume of circulation to a point where progressive changes begin to occur which eventuate in circulatory failure even when the initial fluid loss does not immediately cause death. In studies of hemorrhagic hypotensive shock it has been found necessary to lower the mean arterial pressure to around 50 mm.Hg for 90 minutes and to around 30 mm.Hg for an additional 30 minutes (40) or to between 50 and 60 mm.Hg for 2 to 5 hours (41) (see also table 5) to cause a state of shock in which the arterial pressure progressively declines after reinjection of the withdrawn blood. In the present experiments the arterial pressure declined slowly from 120 to around 50 over several hours, but most of the dogs died within relatively few minutes after the mean arterial had fallen below 50 to 60 mm.Hg. It might be argued on the basis of these arterial pressure readings that slowed blood flow played no part in the shock picture in these dogs. As a result of the temperature studies, however, we are inclined to believe that, following removal of the rubber tubes, the systemic blood flow may have been seriously impaired even when the arterial pressure was still well above the so-called shock level of 50 to 70 mm.Hg.

The dogs in table 1C lost no or very little blood, yet the survival times and the amount of edema were both less than in the dogs placed in recovery cages. Evidently prolonged anesthesia and, particularly, restriction of movement and maintenance of a supine position contributed materially to the ease with which shock was induced by the trauma. This is in agreement with the findings of Swingle et al. (27) in shock induced by pounding the muscle with rubber mallets, with the findings of Kisch (42) in shock induced by intraperitoneal injection

of croton oil, with the observations of Johnson and Blalock (43) on the resistance of dogs to bleeding and plasmapheresis, and with our own observations on shock associated with deep muscle burns (44).

It is our impression also that the course of this form of shock is influenced by the season of the year (39). Thus the dogs studied in May developed more edema and showed greater hyperemia of the lungs and viscera than did those studied during the winter months. This could be due either to a greater hydration of the warm weather dogs, or to a better resistance to the shock processes, thus prolonging life until a greater local fluid loss had occurred.

CONCLUSION. The initial fall of arterial pressure in dogs traumatized by this procedure is induced principally by a combination of reactive vasodilatation in the traumatized legs and initial rapid loss of body fluid into the traumatized tissues. The continuing steady decline of arterial pressure is probably due to a continuing smaller loss of fluid into the traumatized tissues plus the onset of a vicious cycle wherein slowed systemic blood flow results in widespread damage to the vascular system and perhaps also to other tissues. Although no evidence is presented except the small fluid loss in many experiments, it is considered that humoral and nervous factors may very well have contributed to the fatal outcome. Prolonged anesthesia and particularly restriction of movement favor the development of shock.

SUMMARY

An ischemic trauma was produced by application of rubber tubes in the form of a tight continuous spiral bandage from the ankle to the groin of both hind legs of dogs anesthetized with morphine and sodium pentobarbital. Upon release of the tubes after 6 or more hours compression, death occurred in 24 of a first group of 25 minimally anesthetized dogs which were free to move about their cages and in all of a second group of 14 dogs anesthetized for longer intervals and restricted to animal boards in a supine position. The dogs of the first group survived 0.8 to 50 hours after release of the rubber tubes, with an average survival of 11.8 hours. The dogs of the second group survived 2.0 to 20 hours with an average survival of 7.9 hours.

Following the release of the rubber tubes the mean arterial pressure in the dogs of the second group fell rapidly from around 170 to 180 mm.Hg to around 120 to 150 mm.Hg accompanied by a marked rise in heart rate, and a reduction of blood flow in the forepaws, as indicated by subcutaneous temperature records. The mean arterial pressure then declined more slowly to about 50 to 60 mm.Hg, after which death from respiratory or cardiovascular failure ensued within a relatively few minutes. Collapse of the peripheral veins was noted during the period of declining arterial pressure. The hematocrit reading rose rapidly during the first hour after removal of the rubber tubes and continued to rise slightly during the ensuing hours. Depression of activity and sluggish response to stimulation were seen in the lightly anesthetized dogs after release of the limb compression.

The accumulation of fluid in the traumatized extremities was measured by

immersion of the legs separately in a suitable tall narrow vessel before applying the rubber tubes and again after death. After allowing 5 ml./kgm. of dog per extremity for shrinkage of the legs with death and after allowing for the fact that immersion may measure only 91 per cent of the true edema the estimated maximum volume of edema still ranged from 15 to 56 with an average of 41.3 ml./kgm. of body weight in the dogs of the first group and from 3.3 to 33 with an average of 17.6 ml./kgm. of body weight in the second group of experiments. One leg only was traumatized in 7 dogs. Three of these died with an estimated maximum volume of edema of 33, 33 and 55 ml./kgm. The remaining four were killed at approximately 20 hours. The estimated maximum edema in these dogs was 20 to 47 ml./kgm. of dog.

The above observations are not explainable on the basis of a reactive hyperemia in the traumatized legs alone. Accumulation of fluid in the legs is apparently an important factor in the induction of the shock state, but in many of the experiments the volume of edema appears to be insufficient of itself to explain the death. If the operation of humoral or nervous factors are not subsequently demonstrated to play a part in the induction of this type of shock we may have to revise downward our estimates of the quantity of local edema necessary to induce shock, especially in the absence of hemorrhage into the traumatized tissues.

The somewhat shorter survival and lesser edema in the second group of dogs demonstrates the importance of prolonged anesthesia and of restriction of activity in contributing to the ease with which shock with fatal outcome may be induced. Evidence is presented which suggests that dogs studied in the spring resist shock better than do dogs studied during the winter months.

REFERENCES

- (1) EGGLETON, M. G., K. C. RICHARDSON, H. O. SCHILD AND F. R. WINTON. *Quart. J. Exper. Physiol.* 32: 89, 1943.
- (2) BYWATERS, E. G. L. *J. A. M. A.* 124: 1103, 1944.
- (3) GREEN, H. D., N. D. NICKERSON, R. N. LEWIS AND B. L. BROFMAN. *This Journal* 140: 177, 1943.
- (4) GREEN, H. D., R. N. LEWIS, N. D. NICKERSON AND A. L. HELLER. *This Journal* 141: 518, 1944.
- (5) WHIPPLE, G. H., H. B. SHAW AND B. M. BERNHEIM. *J. Exper. Med.* 17: 286, 1913.
- (6) ERLANGER, J., R. GESELL AND H. S. GASSER. *This Journal* 49: 90, 1919.
- (7) ERLANGER, J. AND H. S. GASSER. *This Journal* 49: 151, 1919.
- (8) WHIPPLE, G. H., H. P. SMITH AND A. E. BELT. *This Journal* 52: 72, 1920.
- (9) WERLE, J. M., R. S. COSBY AND C. J. WIGGERS. *This Journal* 136: 401, 1942.
- (10) SWINGLE, W. W., W. KLEINBERG AND H. W. HAYS. *This Journal* 141: 329, 1944.
- (11) CANNON, W. B. AND W. M. BAYLISS. *Med. Res. Committee Reports of the Special Investigation Committee on Shock and Allied Conditions*, no 26, p. 19, London, 1919.
- (12) CANNON, W. B. *Med. Res. Committee. Report on Shock and Allied Conditions*, no. 26, p. 27, 1919.
- (13) CANNON, W. B. *Traumatic shock*. D. Appleton and Co., New York, 1923.
- (14) BLALOCK, A. *Arch. Surg.* 20: 959, 1930.
- (15) PARSONS, E. AND D. B. PREMISTER. *Surg., Gynec. and Obstet.* 51: 196, 1930.
- (16) BLALOCK, A. *Arch. Surg.* 22: 593, 1931.

- (17) FREEDLANDER, S. O. AND C. H. LENHART. *Arch. Surg.* **25**: 693, 1932.
- (18) BROOKS, B. AND A. BLALOCK. *Ann. Surg.* **100**: 728, 1934.
- (19) HOLT, R. L. AND A. D. MACDONALD. *British Med. J.* **1**: 1070, 1934.
- (20) BELL, J. R., A. M. CLARK AND D. P. CUTHBERTSON. *J. Physiol.* **92**: 361, 1938.
- (21) FREEDMAN, A. M. AND H. KABAT. *This Journal* **130**: 620, 1940.
- (22) BEST, C. H. AND D. Y. SOLANDT. *This Journal* **133**: P213, 1941.
- (23) DUNCAN, G. W. AND A. BLALOCK. *Arch. Surg.* **45**: 183, 1942.
- (24) SCHECTER, A. E., M. L. CULLEN AND N. E. FREEMAN. *This Journal* **137**: 710, 1942.
- (25) MANERY, J. AND D. Y. SOLANDT. *This Journal* **138**: 499, 1943.
- (26) ASWORTH, C. T., A. W. JESTER AND E. L. GUY. *This Journal* **141**: 571, 1944.
- (27) EYERSOLE, W. J., W. KLEINBERG, R. R. OVERMAN, J. W. REMINGTON AND W. W. SWINGLE. *This Journal* **140**: 490, 1944.
- (28) QUÉNU, E. *Rev. de Chirurg.* **56**: 204, 1918.
- (29) PAOLUCCI, R. *Arch. ital. di chir.* **21**: 329, 1928.
- (30) FOGLIANI, V. *Riv. di pat. sper.* **9**: 257, 1932.
- (31) WILSON, H. AND N. W. ROOME. *Arch. Surg.* **32**: 334, 1936.
- (32) MYLON, E., M. C. WINTERNITZ AND G. J. DE SÜTÖ-NAGY. *This Journal* **139**: 313, 1943.
- (33) ALLEN, F. M. *Am. J. Surg.* **60**: 335, 1943.
- (34) SCOTT, C. C. AND E. B. ROBBINS. *J. Indiana State Med. Assn.* **36**: 194, 1943.
- (35) SWINGLE, W. W., J. W. REMINGTON, W. KLEINBERG, W. A. DRILL AND W. J. EYERSOLE. *This Journal* **138**: 156, 1942.
- (36) CICARDO, V. H. *Medical Lectures—Buenos Aires*. Reported in *J. A. M. A.* **124**: 454, 1944.
- (37) AUB, J. C., A. M. BRUES, R. DuBos, S. KETY, I. T. NATHANSON, A. POPE AND P. C. ZAMECNIK. *War Med.* **5**: 71, 1944.
- (38) GREEN, H. D. *Circulation: physical principles*. In *Medical Physics*, Otto Glasser, ed. The Year Book Publishers, Inc., Chicago, p. 208, 1944.
- (39) GREEN, H. D. AND G. A. BERGERON. *Surgery* (in press.)
- (40) HUIZENGA, K. A., B. L. BROFMAN AND C. J. WIGGERS. *J. Pharmacol. and Exper. Therap.* **78**: 139, 1943.
- (41) GREEN, H. D. *Anesthesiology* **3**: 611, 1942.
- (42) KISCH, B. *Exper. Med. and Surg.* **2**: 79, 1944.
- (43) JOHNSON, G. S. AND A. BLALOCK. *Arch. Surg.* **22**: 626, 1931.
- (44) GREEN, H. D., R. J. ANTOS AND R. M. DWORKIN. *Proc. Soc. Exper. Biol. and Med.* (in press).
- (45) KATZ, L. N., S. T. KILLIAN, R. ASHER AND S. PERLOW. *This Journal* **137**: 79, 1942.
- (46) ELMAN, R., C. LISCHER AND H. W. DAVEY. *This Journal* **140**: 737, 1944.
- (47) LAWSON, H. *This Journal* **140**: 420, 1943.
- (48) WALCOTT, W. W. *Proc. Soc. Exper. Biol. and Med.* **55**: 272, 1944.
- (49) MCKEE, F. W., C. F. LAYCOCK, T. G. MARTENS AND R. G. NICHOLL. *Surg., Gynec. and Obstet.* **78**: 509, 1944.

FURTHER OBSERVATIONS ON THE WATER AND FAT CONTENT OF THE SKIN AND BODY OF THE ALBINO RAT ON A HIGH FAT DIET¹

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It has been shown (1) that a diet containing 50 per cent fat produces within 70 days a decrease in the water content and an increase in the amount of fat in the skin and body of the albino rat. The present study was undertaken to determine whether similar changes can be induced by the same diet within a shorter time and also whether such changes are quantitatively in proportion to the length of time on the diet.

PROCEDURE. Litter mates of approximately equal weight were selected at weaning and fed for 50 to 70 days on a stock diet similar to the Wistar Dry Ration. They were then placed on the experimental diets. The length of time between weaning and beginning the experiment was so regulated that all the animals at the end of the experiment were the same age as those that had been on the diet 70 days in previous experiments (1). At the end of the preliminary feeding period on the stock diet, one group of 10 animals were fed a high fat diet for one week while their litter mate controls were kept on a stock ration. A second and third group of ten animals each were fed the high fat diet for two and three weeks, respectively, with their litter mates remaining on a stock diet. All the animals were allowed to eat and drink *ad libitum*. The composition of the diets, the experimental procedure and analytical methods were the same as described elsewhere (1).

Method of drying animal tissues. The water content of the skin and body was determined by drying in weighing bottles for 48 hours in an evacuated desiccator over CaCl_2 and then to constant weight over P_2O_5 . This procedure which we have consistently followed (1-3) was the method of choice as we believe it to be more reliable than drying in an oven at 100°-105°C. Two errors are introduced by drying at this temperature: one, a loss of weight due to the volatilization of both fatty and nitrogenous materials; the other, a gain in weight resulting from oxidation (4, 5). While one may at least partially if not completely offset the other, both can be avoided by drying in a vacuum.

Notwithstanding these objections, the water content of animal tissues is sometimes determined by drying in an oven at a high temperature. It was, therefore, deemed advisable to carry out the following experiment to ascertain the extent of the error introduced by this procedure. A rat was decapitated and the entire body ground according to our usual procedure in a meat chopper and then in a corn grinder. These manipulations were carried out in a room saturated with water vapor. The resultant mass has been shown to be

¹ Preliminary report: Federation Proc. 2: 56, 1943.

homogenous (6). Four aliquots were taken from the ground substance. One was dried in a desiccator; one in an oven at 100°–105°C.; and another in a desiccator until it no longer lost weight and then finally to constant weight in an oven. The fourth aliquot was analyzed immediately, and the others after drying, for their fat content. Ten animals were used in this experiment.

The material dried in the oven and also that which was first dried in the desiccator and then in the oven lost more weight than that dried in the desiccator alone. Calculations of the water content based on the loss in weight gave the following values: 64.1 per cent for the material dried in the desiccator; 65.2 per cent for that dried in the oven; and 65.1 per cent for that dried in both desiccator and oven. From these data alone it would appear that more water is removed by drying in the oven. The fat analysis, however, showed that the apparently greater loss of water in the oven-dried tissues was partly due to an error introduced by the volatilization of fat. The fat content of the aliquots dried in the oven was 7.7 per cent and 7.8 per cent, respectively, of the wet weight; whereas the material dried in the desiccator alone contained 9.8 per cent fat which was exactly the same percentage as in the aliquot of the fresh wet tissues. These are average values but it is of interest to note that practically the same difference in the water and fat content of the aliquots dried in the desiccator and oven prevailed in each individual experiment. The difference in the percentage "water" content as determined by the two methods of drying was not precisely the same as the difference in the percentage of fat. This may perhaps be accounted for by oxidation in the oven which partially offset the error introduced by the loss of fat.

The error involved in oven drying is not the same for all the tissues. In another experiment on ten animals the blood, muscle, kidneys, liver, brain and skin were dried first in the desiccator and then in the oven. The changes in the calculated water content after drying in the oven ranged from 0.4 per cent to 2.1 per cent for the various tissues. This was probably due to differences in the amount of fat and in the degree of oxidation in the different tissues.

It was apparent from gross observation of the oven-dried tissues that definite chemical changes had taken place. The material dried in the oven had a dark brown appearance while that dried in the desiccator was the same color as the fresh substance. Upon extraction of the 40 per cent alcoholic solution of the oven dried material, a small amount of gummy substance was thrown out of solution by petroleum ether. This substance was partially soluble in chloroform and 95 per cent alcohol and completely soluble in a mixture of chloroform and alcohol. It did not give a positive test for cholesterol.

RESULTS. Marked changes in the water, protein and fat content of the skin and body were induced within one week by a high fat diet. The analytical data are given in table 1. The percentage of water and of protein was lowered while the percentage of fat showed a definite increase. These differences on the two diets, which persisted without any marked change for three weeks, were observed in each individual experiment. The reduction of the protein content of the body on the fat diet was less than in the skin but was neverthe-

less statistically significant. Table 2 is presented to facilitate comparison of week to week changes induced in the composition of the skin and body on the high fat diet.

TABLE 1

*Percentage fat, water and protein content of the skin and body of albino rats on a high fat and on a stock ration**

	MALES						FEMALES					
	High fat diet			Stock diet			High fat diet			Stock diet		
	Weeks on diet											
	1	2	3	1	2	3	1	2	3	1	2	3
	%	%	%	%	%	%	%	%	%	%	%	%
Fat in skin.....	13.4	12.9	14.7	6.6	7.3	6.6	17.5	18.3	22.6	11.0	10.5	10.5
H ₂ O in skin.....	57.1	56.9	57.1	61.7	61.8	61.9	50.1	52.2	51.2	57.4	59.5	59.3
Fat in body.....	18.5	17.8	17.2	10.5	10.0	9.4	18.5	19.3	18.2	10.5	12.6	9.9
H ₂ O in body.....	57.5	56.9	57.1	63.1	61.8	61.9	57.4	56.4	57.2	64.3	61.7	63.4
Protein in skin (N× 6.25).....	25.0	25.0	24.4	29.4	29.4	30.0	22.5	22.5	22.5	26.9	25.6	25.0
Protein in body (N× 6.25).....	19.4	17.5	18.8	20.6	19.4	19.4	17.5	16.8	17.5	19.4	18.1	18.8

* Each value in the table is an average of five experiments.

TABLE 2

Differences in the percentage fat, water and protein in the skin and body resulting from a high fat intake

WEEKS ON RATION	FAT IN SKIN	H ₂ O IN SKIN	FAT IN BODY	H ₂ O IN BODY	PROTEIN IN SKIN (N×6.25)	PROTEIN IN BODY (N×6.25)
Males						
1	+6.8	-4.4	+8.0	-5.6	-4.4	-1.2
2	+5.6	-4.9	+7.8	-5.5	-4.4	-1.9
3	+8.1	-4.8	+7.8	-6.4	-5.6	-0.6
Females						
1	+6.5	-7.3	+8.0	-6.9	-4.4	-1.9
2	+7.8	-7.3	+6.6	-5.3	-3.1	-1.3
3	+10.1	-8.1	+8.3	-6.2	-2.5	-1.3

A plus sign indicates a higher percentage on the fat ration; a minus sign a lower percentage.

From these data it is apparent that feeding a high fat diet produced the same qualitative effect on the skin and body within one week as was observed previously (1) after 70 days on the diet. Quantitatively, however, the effect was more pronounced with the longer feeding. At the end of 70 days there was an increase in the fat content of the skin of the males and females over the controls

of 11.0 and 19.6 per cent respectively, whereas in three weeks the increase was 8.1 and 10.1 per cent. The water content was reduced 8.9 and 11.9 per cent, respectively, in 70 days but only 4.8 and 7.3 per cent in three weeks. These more pronounced changes with longer feeding evidently took place between the third and tenth week.

The sex differences in the water and fat content of the skin reported elsewhere (1) were observed also in the present experiments (table 1). Since these differences obtained on both the stock and high fat diets and also in other experiments on a high carbohydrate diet, it may be concluded that they were not dependent on the composition of the diet.

CONCLUSIONS

A high fat diet induced a definite increase in the fat content and a decrease in the percentage of water and of protein in the skin and body of the albino rat within one week.

These changes were qualitatively the same although quantitatively not as large as those previously observed at the end of 70 days on the diet.

The same sex differences in the composition of the skin obtained on a high fat diet as on a stock and high carbohydrate diet.

Drying of the animal tissues in an evacuated desiccator is a more reliable method for determining the water and fat content than drying in an oven at 100-105°C.

REFERENCES

- (1) HALDI, J., G. GIDDINGS AND W. WYNN. This Journal **135**: 392, 1942.
- (2) HALDI, J. AND G. GIDDINGS. This Journal **128**: 537, 1940.
- (3) HALDI, J., G. GIDDINGS AND W. WYNN. This Journal **141**: 83, 1944.
- (4) BENEDICT, F. G. AND C. R. MANNING. This Journal **13**: 309, 1905.
- (5) BENEDICT, F. G. AND C. R. MANNING. This Journal **18**: 213, 1907.
- (6) HALDI, J., G. BACHMANN, W. WYNN AND C. ENSOR. J. Nutrition **18**: 399, 1939.

THE EFFECTS OF POTASSIUM ON THE SYNTHESIS OF ACETYLCHOLINE IN BRAIN

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An increased synthesis of acetylcholine (ACh) in the presence of high concentrations of potassium has been reported by Mann, Tennenbaum and Quastel (1939), using slices of adult rat cerebrum. The probable connection of K^+ with some phase or phases of carbohydrate metabolism has recently become increasingly evident. In view of a probable dependence of ACh synthesis *in vivo* on normal carbohydrate metabolism (Welsh, 1943), it was of interest to confirm and extend the work of Mann et al. (1939). The present paper offers results of experiments with aerobic and anaerobic synthesis of ACh by infant and adult rat brain slices in media containing different concentrations of KCl. Infant tissues were used as well as adult since Sykowski, Fazekas and Himwich (1939) reported a greater production of ACh by infant than adult rat brain. Several possible mechanisms for the observed effects of potassium are discussed.

METHODS. Rats of the Sprague-Dawley albino strain were used. Adult rats were both males and females; the infant rats used were all males and were less than 24 hours old.

The rats were beheaded; the whole brain, including cerebrum, cerebellum, brainstem and medulla, was removed. Brains of adults were split sagittally, those of infants were left whole; these were rinsed, blotted with filter paper to remove excess moisture, and sliced as thin as possible. One half of an adult brain or 3 infant brains were used in each experiment. The weighed tissue was placed in 125 cc. flasks in 3 cc. of medium per 100 mgm. of tissue. Suitable gas mixtures (95 per cent O_2 -5 per cent CO_2 for aerobic experiments; 95 per cent N_2 -5 per cent CO_2 for anaerobic experiments) were bubbled through the medium in the flasks for 10 or 20 minutes; the flasks were sealed, shaken in a constant temperature bath for 1 hour and 15 minutes at $37^\circ C.$, and the contents were extracted for "free" or total ACh.

The normal bicarbonate-Locke used consisted of NaCl, 0.13 M; KCl, 0.004 M; $CaCl_2$, 0.002 M; and $NaHCO_3$, 0.025 M. The modified solutions contained KCl 0.027 M, 0.05 M, 0.075 M, or 0.10 M, with a corresponding decrease in NaCl to maintain isotonicity. In all experiments glucose was added to give 1 part in 500. Eserine sulphate 1 part in 5,000 was added at the start or, in certain of the anaerobic experiments, between two 10-minute periods of bubbling the N_2 - CO_2 mixture through the fluid in the flask.

The extraction of "free" ACh (that part of the total ACh readily extractable in water, in the presence of adequate amounts of eserine and without employing a protein denaturant) was done by centrifuging the contents of a flask, the supernatant fluid being decanted and assayed for its ACh content. To extract

the total ACh, the tissue slices were ground with sand, acidified in the original fluid to pH 3 (using Congo red paper as an indicator) with normal HCl, let stand one hour, then partially neutralized to pH 5-6 with normal NaOH, and centrifuged. Further dilution with buffered solutions at the time of assay raised the pH to 7 or 7.5.

The isolated ventricle preparation of the mollusc, *Venus mercenaria*, was used for assaying the extracts. Records were obtained as in figure 1. The depressions of the beat caused by known amounts of ACh were compared with those caused by different amounts of extract. Values are expressed in gamma of ACh

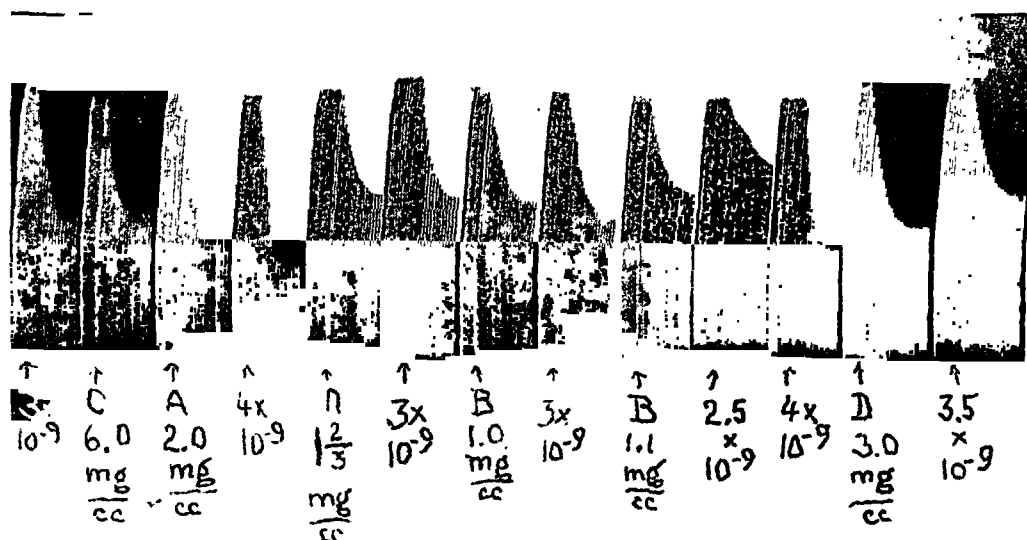


Fig. 1. A portion of an assay record using isolated heart of *Venus mercenaria*. At 3×10^{-9} , 4×10^{-9} , etc., are records of the depression produced by these known concentrations of acetylcholine. At C, 6.0 mgm./cc., A, 2.0 mgm./cc., etc., are records of the depression produced by extracts containing the active material from 6.0 mgm., 2.0 mgm., etc., of brain tissue per cubic centimeter of fluid bathing the heart. Test solutions allowed to remain in bath approximately 1 min. Between tests heart was washed repeatedly and allowed 5 to 10 min. to recover original amplitude.

A = adult, free ACh; B = adult, total ACh; C = infant, free ACh; D = infant, total ACh. Conditions of experiment: anaerobic; 0.05 M K^+ ; eserine 1:5000 from time tissues were weighed.

per gram of moist tissue. Stock acetylcholine chloride was made up in five per cent NaH_2PO_4 , sealed in ampoules, heated in a water bath at $100^\circ C$ for 10 minutes and stored at a low temperature until used. The stock solution contained one part by weight of the base in 1000 parts of water, and results are expressed in terms of equivalent weights of the base, not the salt.

RESULTS. Estimated values of ACh present in whole rat brain extracted immediately after decapitation are as follows: adult brain contains about 0.5 γ free ACh, and 1.0-1.5 γ total ACh/gram tissue; infant brain contains 0.1-0.2 γ free, and 0.4-0.6 γ total ACh/gram tissue (Welsh and Hyde, 1944; unpublished observations).

The estimated values of free and total ACh obtained from adult and infant rat brain slices incubated in bicarbonate-Locke solutions with different potassium concentrations are given in table 1. From this table it will be seen that at 0.004 M K^+ , the concentration in normal bicarbonate-Locke, least ACh is synthesized aerobically. The adult brain slices contain an average of 5.1 γ

TABLE 1
Acetylcholine synthesis in normal and modified bicarbonate Locke's solution
(ACh in gamma per gram)

CONC. KCl	AEROBIC				ANAEROBIC							
					Eserine from start				Eserine during N_2CO_2			
	Adult		Infant		Adult		Infant		Adult		Infant	
	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total
0.004M	1.0	6.0	0.2	1.5	1	2	0.3	1.5	0.9	2.7	0.03	0.6
	0.3	1.4	0.04	0.4	1	2.6	0.3	1.6	0.8	1.2	0.33	1.2
	0.5	5.0	0.15	0.6					0.5	2.0	0.05	0.8
	1.4	8.0	0.24	2.0								
Av.....	0.8	5.1	0.16	1.1	1	2.3	0.3	1.5	0.7	2.0	0.14	0.9
0.027M	6	12	0.5	1.0								
	10	15	0.5	1.0								
	7.5	13	0.5	2.0								
Av.....	8	13	0.5	1.3								
0.050M	10	8	1	3	1.8	2.8	0.5	1.6	0.6	1.0	0.25	0.7
	14	15	1.6	3.3	2	4	1	2	0.6	1.2	0.2	0.6
	10	10	1	2.5					0.5	1.25	0.3	0.4
	13.3	13.3	1.2	2.3								
Av.....	12	12	1.2	2.8	1.9	3.4	0.7	1.8	0.6	1.2	0.25	0.6
0.075M	5.0	6.7	0.5	1.0								
0.100M	3.5	7.5	0.7	2.3								
	2.0	2.5	0.8	1.0								
	4.0	4.2	1.7	2.2								
	2.0	3.3	0.7	1.0								
	3.3	5.0	0.3	1.3								
Av.....	3.0	4.0	0.9	1.3								

total ACh per gram, the infant, 1.1 γ /gram. These values are 6 and 7 \times the values for free ACh present at this K^+ concentration; the values for free ACh in both infant and adult are not much larger than those before synthesis. Since before incubation values for total ACh average 2-3 \times those for free ACh, this suggests that at this low K^+ concentration, bound ACh is synthesized but little or no free ACh is released.

The rate of synthesis is increased in a medium containing 0.027 M K^+ . Adult brain slices at this concentration contain an average of 13 γ total ACh/gram, a considerable increase over the amount synthesized at 0.004 M K^+ . The average infant value for total ACh at 0.027 M K^+ is only slightly larger than that at normal K^+ . The values for free ACh in both infant and adult are considerably larger than those in normal K^+ , suggesting that at the higher K^+ some of the bound or combined ACh is being released.

At 0.05 M K^+ the value for total ACh is, in the adult, about the same as at 0.027 M K^+ , and in the infant, the highest at any K^+ concentration. Thus synthesis is maximal at a concentration of K^+ between 0.027 and 0.05 M. It is interesting to note that 0.05 M is very nearly the concentration of K^+ inside frog nerve cells (Fenn, Cobb, Hegnauer and Marsh, 1934). At 0.05 M K^+ all the ACh in the adult brain slices is present as free ACh, due presumably to complete release or replacement of bound ACh.

A single set of determinations at 0.075 M K^+ showed a decline in synthesis by both infant and adult tissue. This decline is further shown by the results of experiments at 0.10 M K^+ , where the values for total ACh are approximately equal to those at 0.004 M K^+ . The values for free ACh at this high K^+ concentration are almost as large as those for total ACh in both infant and adult tissue, indicating again replacement or release of bound ACh in the presence of high K^+ .

Anaerobic series. The values obtained for synthesis under anaerobic conditions are also contained in table 1. A few experiments were carried out in which eserine was present in the fluid in which the tissues were weighed. Since this meant that the ACh synthesized during about 10 minutes of aerobic conditions was being preserved, subsequent experiments were carried out in which the eserine was added to the flasks in which nitrogen was being bubbled through so that of the free ACh only that actually released under anaerobic conditions was preserved. The determinations of ACh synthesized anaerobically were made for only two concentrations of KCl: 0.004 M, the concentration in normal bicarbonate-Locke, and 0.05 M KCl, the concentration at which synthesis and release were greatest under aerobic conditions.

From table 1 it will be seen that at 0.004 M K^+ the total ACh in adult slices under partially anaerobic conditions is less than that under aerobic conditions, while the infant total ACh is slightly larger than that found under aerobic conditions. Values for free ACh are similar to those found under aerobic conditions at this concentration. At 0.05 M K^+ there is very little increase in ACh synthesized by infant and adult brain slices.

In the experiments in which eserine was added while nitrogen was being bubbled through the tissue medium, a more accurate test of the possible effects of K^+ on anaerobic synthesis was made. Of the free ACh, only that actually released was preserved under these conditions. At 0.004 M K^+ values are similar to, but slightly smaller than, those obtained under the partially anaerobic conditions where eserine was added to the tissues before the nitrogen was bubbled through. At 0.05 M K^+ it is seen that the results are very different from

those obtained with aerobic synthesis. Instead of the large increase in ACh synthesized aerobically in high concentrations of K^+ , there is, if anything, slightly less ACh produced anaerobically at 0.05 M K^+ than at 0.004 M K^+ . These findings are in agreement with observations of the effects of high K^+ on aerobic and anaerobic glycolysis in brain (Ashford and Dixon, 1935; Dickens and Greville, 1935).

DISCUSSION. The results of synthesis of ACh by adult rat brain slices recorded above, using different concentrations of K^+ , agree in most respects with those of Mann, Tennenbaum and Quastel (1939). In a solution with 7-8 \times the amount of K^+ of normal bicarbonate-Locke, synthesis is at a maximum; at about 12 \times normal K^+ the release of bound ACh is at a maximum; a further increase in K^+ inhibits synthesis.

The actual values we obtained are not as high as those of Mann et al., but this may be partly due to differences in technique. They used slices of whole cerebrum, we used slices of all parts of the brain. Their values are expressed in terms of AChCl; ours are as equivalents of the free base. With their assay object, the leech muscle, any response to high K^+ would be additive to the ACh response, while with the Venus heart the effect of high K^+ is opposite that of ACh.¹

In addition to confirming this work it was also shown that the effect of added K^+ on synthesis by incubated slices of infant brain gave results essentially like those obtained with adult tissue. The infant brain contains much less ACh at the start than the adult brain, and the dry weight of the infant rat brain is only 10 per cent of the total wet weight, while the dry weight of the adult brain is 22 per cent of the wet weight (Koch and Koch, 1913).

Anaerobic experiments using normal bicarbonate-Locke confirmed the earlier observations of Quastel, Tennenbaum and Wheatley (1936) and Mann, Tennenbaum and Quastel (1938) that little, if any, synthesis of ACh by brain slices occurs in the absence of O_2 . Under anaerobic conditions less ACh, if anything, was formed at 0.05 M K^+ than at normal K^+ .

We failed to find, under any of the conditions we tried, more free ACh formed by newborn than by adult rat brain slices, as reported by Sykowski, Fazekas and Himwich (1939), even though in a few experiments (not recorded under the results given above) we tried phosphate-Locke as used by these investigators. If calculated on a basis of dry weight the values for infant free ACh in the phosphate-Locke experiments were about the same as those for adult; the considerably larger infant yields reported by Sykowski et al. could not be confirmed, however. There are several differences in technique which might help explain this.

Mann, Tennenbaum and Quastel (1939) explained their results largely on the basis of changes in permeability of the nerve cell membrane produced by K^+ ; and also by the breakdown of the "combined" form of the ester. A reciprocal action of K^+ and ACh in tissues is well known. A liberation of ACh by KCl has been observed in experiments with several different organs (Beznak,

¹ With neither the leech muscle nor the Venus heart does potassium, in the concentrations used, seriously interfere with the assay.

1934; Brown and Feldberg, 1936 and 1936-37; Chute, Feldberg and Smyth, 1940). Similarly ACh releases K^+ (Dulière and Loewi, 1939; Cicardo and Moglia, 1940). However, it is difficult to see how increased permeability and release of bound ACh could account for the *very much* greater ACh synthesis per unit of weight and time at 0.027-0.05 M K^+ . It is believed that K^+ has one or more additional actions affecting the synthesis of ACh.

There is growing evidence that K^+ plays an important rôle in the metabolism of the nerve cell. This is seen in its effects on respiration and glucolysis of brain. Recently there is evidence of a rôle of K^+ in carbohydrate metabolism and specifically in certain phases of the phosphorylation process. Nachmansohn and Machado (1943) have secured a large production of ACh in cell free extracts of rat brain upon the addition of choline chloride, eserine sulphate, sodium fluoride and adenosine triphosphate (even under anaerobic conditions), thus showing that the final step or steps in synthesis of ACh may derive energy from the splitting of ATP. Nevertheless there is good evidence that the synthesis of ACh by tissues normally requires an adequate supply of glucose and oxygen (Quastel et al., 1936; Mann et al., 1938; Welsh, 1943). It may be assumed that in tissues all of the factors responsible for normal carbohydrate metabolism are important in determining the rate of synthesis of ACh. K^+ is apparently one of these.

Ashford and Dixon (1935), and later Dickens and Greville (1935), found that aerobic glycolysis in brain slices was greatly increased at K^+ concentrations between 0.02 and 0.1 M. They also showed that anaerobic glycolysis was decreased by high K^+ . It is to be noted that the production of ACh under aerobic and anaerobic conditions is affected similarly by K^+ . More recently it has been suggested that K^+ has an effect on some phosphorylation phase or phases of carbohydrate metabolism (Verzár and Somogyi, 1939; Pulver and Verzár, 1940; Lasnitzki, 1940; Boyer, Lardy and Phillips, 1942 and 1943). These latter authors have evidence to show that K^+ has a specific effect on the phosphorylation of the adenylic system in muscle. The inhibiting action of adenosine triphosphatase by K^+ (Mehl and Sexton, 1943) is also to be noted.

Nachmansohn and Machado (1943) tried 0.02-0.06 M K^+ on their extracts of rat brain and found no effect on the formation of ACh. They concluded that increased K^+ is not directly involved in ACh formation and that Mann et al. were correct in ascribing the action of K^+ to changes in permeability. However, if high K^+ favors the formation of ATP no action would be expected in Nachmansohn and Machado's experiments where adequate supplies of ATP were already present.²

K^+ may be considered to have several rôles in the synthesis and release of ACh: 1, affecting permeability of the cell membrane; 2, replacement of ACh on a "precursor" or synthesizing enzyme (perhaps Nachmansohn's "choline acetylase"); 3, one or more steps leading up to the formation and breakdown

² Recently Nachmansohn (personal communication) has shown that K^+ is required by the "choline acetylase" system. Between 0.01 and 0.02 M KCl there is a marked increase in ACh-formation. Higher concentrations of KCl, up to 0.16 M, result in only slightly more ACh being formed.

of ATP to provide energy for ACh formation. The physiological actions of increased K^+ on nerve cells are well known (see Fenn, 1940). To what extent they are direct actions and to what extent indirect, by way of the ACh mechanism, remains to be seen.

SUMMARY

The effect of increased potassium on the synthesis and release of acetylcholine (ACh) by slices of newborn and adult rat brain, under aerobic and anaerobic conditions, was studied. The earlier findings of Mann, Tennenbaum and Quastel were confirmed, namely that at 7 to 8 \times the K^+ of normal Locke's solution the synthesis of ACh by brain slices in O_2 was maximal; at about 12 \times K^+ , the release of ACh was maximal; further increase in K^+ resulted in a decrease in ACh formation.

In addition to confirming Mann et al., it was found that slices of infant brain behaved essentially as adult except that the yields of ACh were not as great. Also, that under anaerobic conditions very little ACh is synthesized at 0.004 M K^+ and increasing the K^+ to 0.05 M causes a possibly significant slight decline in total ACh.

Mann et al., considered their observed K^+ effects due primarily to changes in cell permeability. Recent observations suggest that K^+ plays a rôle in carbohydrate metabolism, and that high K^+ may favor the formation of energy-rich phosphate compounds shown by Nachmansohn to supply energy for the synthesis of ACh. Thus more than one action of K^+ is probably responsible for its observed effects on ACh formation and release.

REFERENCES

- ASHFORD, C. A. AND K. C. DIXON. *Biochem. J.* 29: 157, 1935.
 BEZNAK, A. B. L. *J. Physiol.* 82: 129, 1934.
 BOYER, P. D., H. A. LARDY AND P. H. PHILLIPS. *J. Biol. Chem.* 146: 673, 1942; *J. Biol. Chem.* 149: 529, 1943.
 BROWN, G. L. AND W. FELDBERG. *J. Physiol.* 86: 290, 1936; *J. Physiol.* 88: 265, 1936-37.
 CHUTE, A. L., W. FELDBERG AND D. H. SMYTH. *Quart. J. Exper. Physiol.* 30: 65, 1940.
 CICARDO, V. H. AND J. A. MOGLIA. *Nature* 145: 551, 1940.
 DICKENS, F. AND G. D. GREVILLE. *Biochem. J.* 29: 1468, 1935.
 DULIÈRE, W. AND O. LOEWI. *Nature* 144: 244, 1939.
 FENN, W. O. *Physiol. Rev.* 20: 377, 1940.
 FENN, W. O., D. M. COBB, A. H. HEGNAUER AND B. S. MARSH. *This Journal* 110: 74, 1934.
 KOCH, W. AND M. L. KOCH. *J. Biol. Chem.* 15: 423, 1913.
 LASNITZKI, A. *Nature* 146: 99, 1940.
 MANN, P. J. G., M. TENNENBAUM AND J. H. QUASTEL. *Biochem. J.* 32: 243, 1938; *Biochem. J.* 33: 822, 1939.
 MEHL, J. W. AND E. L. SEXTON. *Proc. Soc. Exper. Biol. and Med.* 52: 38, 1943.
 NACHMANSOHN, D. AND A. L. MACHADO. *J. Neurophysiol.* 6: 379, 1943.
 PULVER, R. AND F. VERZÁR. *Nature* 145: 823, 1940.
 QUASTEL, J. H., M. TENNENBAUM AND A. H. M. WHEATLEY. *Biochem. J.* 30: 1668, 1936.
 SYKOWSKI, P., J. F. FAZEKAS AND H. E. HIMWICH. *This Journal* 127: 381, 1939.
 VERZÁR, F. AND J. C. SOMOGYI. *Nature* 144: 1014, 1939.
 WELSH, J. H. *J. Neurophysiol.* 6: 329, 1943.
 WELSH, J. H. AND J. E. HYDE. *J. Neurophysiol.* 7: 41, 1944.

THE PRODUCTION OF SHOCK BY CALLICREIN¹

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Much of the shock literature during the past twenty years has been devoted to the problem of whether or not the liberation of toxic chemical materials is involved in the production of shock. (For recent reviews of the shock literature, cf., 1, 2.) While the toxin liberation theory is most attractive from many aspects, it has been lacking in conviction, not only because of the failures to obtain direct evidence in its support, but also because of our lack of knowledge of prototypes of the hypothetical toxic substance.

Few known substances that occur in mammalian tissues could fulfill this rôle of a toxin. Histamine has been studied extensively in this connection, and its importance in shock has been effectively discounted (1, 2). Another substance with shock producing properties which has previously escaped much attention is callicrein. Studies were, therefore, undertaken on the relationship of callicrein to shock.

The hypotensive substance known as callicrein (or kallikrein) was first discovered (3) and systematically studied (4-11) by Frey, Kraut, Werle and co-workers. It is present in most mammalian tissues in small concentration (6), and is especially abundant and readily demonstrated in urine (11-14) and pancreas (6, 7, 15, 16), saliva and salivary glands (17, 18, 19) and throughout the intestinal tract (20). Callicrein is found in the free, active form in urine, saliva (17) and possibly salivary glands (19, 21), whereas that found in the pancreas (21) and intestinal tract (22) is liberated from a bound-inactive form by the action of proteolytic enzymes (23). Relatively large amounts of the inactive callicrein occur in serum (10, 24), and can be converted to the hypotensive form by acidification, treatment with acetone or proteolytic destruction of the inactivator (10, 13, 24).

At least two different substances occurring in the body inactivate callicrein by combining with it. One of these inactivators occurs in the gamma-globulin fraction of serum (25, 26), while the tissue inactivator is a basic protein (27) especially abundant in cattle lymph nodes and spleen (8). Incubation of callicrein with either inactivator results in a combination that is no longer hypotensive. The combination can be split with release of active callicrein by acidification. The reaction between callicrein and the inactivator depends upon the pH of the medium (8, 26); alkaline solutions favor the formation of the inactive complex, while acid reactions prevent it.

Callicrein can be prepared most readily from urine (9, 28, 29) or pancreas

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(30). It has never been isolated in pure form, and various chemical studies (31, 32) of purified preparations have not established its chemical nature. Certain criteria (20) will distinguish callicrein from other hypotensive substances; callicrein is *a*, heat labile; *b*, non-dialyzable through cellophane; *c*, inactivated by incubation with serum, and it will *d*, increase the pulse pressure, and *e*, cause contraction of the isolated dog intestine (33, 34, 35).

The hypotension resulting from callicrein is not a cardiac phenomenon, but is attributed to an effect on the tonus of the blood vessels (36) and the resulting splanchnic and peripheral vasodilatation (5, 37, 38, 39). The coronary arteries (40) as well as the vessels in the lungs, brain, etc., (5, 37) are also dilated. It affects the blood pressure only when injected intravenously (31), into a related species (41, 42), and some of the pharmacological effects of small intravenous doses have been described (43-49); it is antagonistic to adrenaline (14, 15, 28, 31, 32, 50, 51). Indirect evidence indicates that callicrein increases capillary permeability (52-55).

The experiments described in this report show that callicrein will reproduce the many features of the shock syndrome. Its administration led to a hypotension, increased heart rate, decreased cardiac output, hemoconcentration, plasma loss, anoxia, acidosis and death from respiratory failure. As a natural constituent of mammalian blood and tissue, it possesses the attributes of a shock producing material that might prove to be of fundamental importance in the etiology of shock.

EXPERIMENTAL The callicrein used in these studies was prepared from normal human urine (collected and processed under toluene or xylene) by adsorption on a uranium acetate precipitate and elution with diammonium phosphate as described by Kraut et al. (9). After exhaustive dialysis of the eluate, the callicrein was adsorbed on benzoic acid and obtained as a dry powder by removal of the benzoic acid with alcohol-ether (9). This powder, which usually contained 3.3 Frey units per milligram, was redissolved in M/40 NaHCO_3 and administered intravenously. Some experiments were carried out with the dialyzed eluate made isotonic with sodium chloride. In order to determine to what extent the principal shock effects observed may have been due to impurities, control experiments were conducted with material in which the callicrein had been inactivated.

Some of the experiments were carried out on normal, unoperated and unanesthetized dogs; other dogs were anesthetized by an initial intravenous dose of 32.5 mgm. nembutal per kgm., and additional amounts were given as needed. Little or no additional anesthesia was needed after the callicrein administration (49). Minor operative procedures were carried out in unanesthetized animals under local procaine treatment. About fifty dogs were used in the experiments described herein and in most cases their previous history was unknown. Seventy-five per cent of the dogs weighed between 10 and 15 kgm.—the average was 13.5 kgm. Seventy per cent of the dogs were male. Experiments were also carried out on 4 dogs which were splenectomized three to four weeks before being used. A protocol giving some details of all the experiments herein described has been included.

Blood samples were withdrawn from the jugular vein or from a catheter inserted through the jugular vein into the vena cava in the region of the heart. Samples taken for analysis of the blood gases were removed under oil and analyzed immediately. Heparin was used as an anticoagulant for the blood samples. Loss of blood, either by hemorrhage or sampling, was not an important

Protocols

DOG AND EXPT. NO.	WEIGHT	SEX	NEU- BU- TAL	BLOOD SAMPLES FROM JUGULAR OR VENA CAVA	UNITS OF CALLI- CREIN ADMINIS- TERED	DURA- TION OF ADMIN- ISTRA- TION	SURVIVAL AFTER CALLICREIN	BLOOD PRES- SURE AT START	REMARKS
	<i>kgm.</i>					<i>minutes</i>	<i>hours</i>		
1	15	F	+	J	1,400	450	7½	150	
2	15.5	F	+	J	760	160	6	170	
3	13.6	M	+	J	160	45	4½	140	
5	14.3	F	+	J	200	25	1¾	120	
6	14.6	M	-	J	400	30	4½		
7	14.0	M	-	J	200	30	Between 5-20 hrs.		
9	12.0	M	-	J	200	10	12		
10	10.0	F	-	J	400	90	5½		
13	22	M	+	J	400	10	6½		
20	16.0	M	+	VC	200	5	12¾	155	
24	16	M	+	VC	200	5	4½	120	
27	11.3	M	-	VC	200	5	6		
29	15	M	-	VC	200	5	4.5		
31	15	F	-	VC	130	45	Lived		
32	12	M	-	VC	200	45	4		
35	11.1	M	-	VC	200	30	7½		
38	19	M	-	VC	150	50	11½		Splenect. 3 wks.
39	8.5	M	-	VC	200	40	4		Splenect. 3 wks.
40	12	M	-	VC	150	40	29		Splenect. 3 wks.
41	12.5	M	-	VC	200	60	22		200 un. call. at 27 hrs.
43	8.5	M	+	VC	200	20	12½	145	Splenect. 4 wks.
46	13.4	F	-	VC	400	30	36		100 un. call. at 20 hrs.
									200 cc. serum 1 hr. after callicrein
									250 cc. dog serum 4 hrs. after call.
									Comatose 24 hrs. before death
54	11.8	M	+	VC	200	5	4½	135	
56	15	M	+	Heart	100-150	5	8	135	
57	11.4	F	+	Heart	100-150	5	10	140	
60	10.0	M	+	Heart	100-150	5	11½	140	Resistance breathing

complication; the total amount removed in any experiment was usually not more than 0.3 to 0.4 per cent of the body weight. In experiments in which the plasma volume was determined or in which complete analyses of the inorganic and organic constituents were made, the total blood withdrawn was greater than this but did not exceed 1 per cent of the body weight.

General Effects of Callicrein. The intravenous injection of 200 units of callicrein killed all anesthetized dogs. This was 60 mgm. of the usual urinary preparation, or the average amount of callicrein excreted daily by a normal human (11). No essential differences were noted when the total dose was infused slowly over a period of an hour, or when it was injected as rapidly as within 5 minutes; in either case, a time interval was necessary before the animal died, and this was true even when ten times this usual dose was given. There was a great variation among dogs in their response to callicrein; the most rapid death occurred in 1.5 hours, the slowest in about 12 hours, and death occurred between 4 and 7.5 hours after starting the callicrein administration in 70 per cent of the experiments. The unanesthetized dogs were more resistant to the lethal action of callicrein, and 6 out of 18 dogs recovered after receiving the usual 200 units. The survival time of those dying averaged $6\frac{1}{4}$ hours as compared with $5\frac{1}{4}$ hours for anesthetized dogs.

The effects of callicrein in both anesthetized and unanesthetized dogs were similar, with two notable exceptions. a. Unanesthetized dogs responded to intravenous callicrein with salivation, vomiting, retching, and strong intestinal contractions and evacuations (33, 34, 35). The animals became very thirsty, but vomited any water given them. Anesthetized dogs showed only mild intestinal activity. b. The rectal temperature rose after the administration of callicrein to unanesthetized dogs (46, 47), but remained the same or fell slowly in anesthetized animals. In both types of experiments there appeared to be a cessation or marked diminution of urine output. Defecation occurred in all dogs, and a bloody diarrhea developed in many.

Within $\frac{1}{2}$ hour of the start of the infusion of callicrein into unanesthetized dogs, they became very listless and depressed. The dogs occasionally moved their eyes, but looked tired, sick and apathetic. This condition usually lasted for several hours, and then either became progressively worse or the dog showed a temporary improvement. The progressive deterioration resulted in a condition of stupor or unconsciousness. The dog had a blank, vacant stare, and did not blink or move when approached. The corneal reflex was present, but there was no response to painful stimuli. Death was immediately preceded by the gasping symptoms of respiratory failure. The heart continued beating after respirations stopped, but the heart contained little blood. Many dogs showed a temporary recovery from the initial depression effects, the improvement lasting for several hours. There was every indication from outward appearances that the dog had recovered, though he probably was not as active or lively as normally. Eventually, however, the progressive deterioration supervened and led to death in the manner previously described.

Physiological Responses to Callicrein. Blood pressure changes. Changes in the mean arterial pressure were measured in anesthetized dogs by a mercury manometer connected by a citrate solution to a carotid cannula. Although individual responses were sometimes quite different, the general pattern of the response to callicrein is illustrated by several representative tracings in figure 1. These curves show the initial precipitous drop in pressure to 40–60 mm. Hg and the

two secondary rises usually occurring in about 5 minutes and 30 minutes after the start of the callicrein administration and in spite of its continued administration. After the second rise to 60–90 mm. Hg, the pressure fell gradually over a period of hours until death, or it fell to a level of 35–40 mm. Hg and remained there for several hours before death. A premortal rise in pressure, with large oscillations of the manometer, coincided with the gasping signs of respiratory failure.

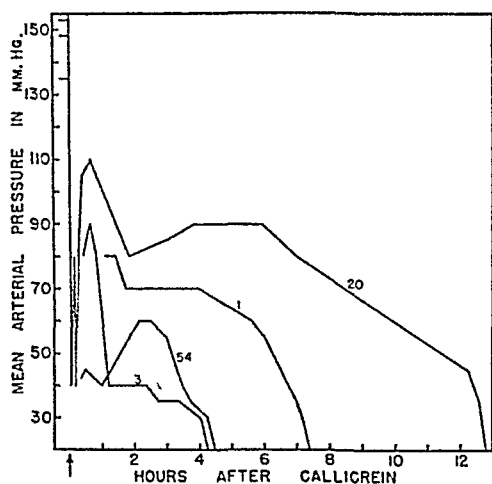


Fig. 1

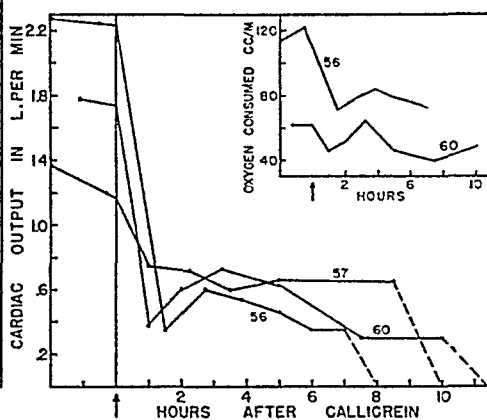


Fig. 2

Fig. 1. The effect of callicrein on the mean arterial blood pressure.

Callicrein administration was started at the arrow. Changes during the first half-hour are shown for no. 20 only. Similar changes in the others are as follows:

No. 1—160, 120, 150, 40, 65, 80

No. 3—150, 60, 95, 50, 80

No. 54—135, 50, 70, 40, 45

Fig. 2. Changes in cardiac output and oxygen consumption following callicrein administration.

Callicrein was given at the arrow. Each line has been extended to the time of death. The nembutal was administered 2½ hours before the callicrein in experiments no. 56 and no. 60, and 4 hours in experiment no. 57. The blood pressures existing at each measurement of the cardiac output (shown by the experimental points on the curves) were:

No. 56—135, 105, 40, 75, 75, 55, 45, 35

No. 57—140, 125, 50, 70, 80, 85, 50

No. 60—140, 55, 65, 75, 135, 75, 60

No. 60 was breathing against a resistance during the interval from 1½ to 5¼ hours after callicrein.

The pressure changes were similar whether the callicrein was given rapidly or slowly, or when the amount administered was ten times the usual dose. It is probable that part of the secondary rise in pressure was due to "neutralization" of the callicrein by adrenaline (31, 32). Death occurred in 1½ hours in a dog that showed little or no secondary rise in pressure after callicrein had decreased it to 40 mm. Hg.

Death resulting from the administration of callicrein was not the result of low blood pressure *per se*. In experiment 3, 160 units of callicrein were infused

over 45 minutes and during this time, the blood pressure fell below 80 mm. Hg for only 20 to 25 minutes. However, enough damage was effected during this time so that without further treatment, the blood pressure fell slowly during the next $3\frac{1}{2}$ hours until death occurred.

In one experiment the callicrein was infused through the lymphatics by cannulation of a lymph vessel in the right popliteal space. The blood pressure response was a slow gradual fall over 1 hour from 150 to 60 mm. Hg, where it remained over 5 hours before accidental death; the precipitous pressure changes characteristic of intravenous callicrein did not occur, but other effects (such as a bloody mucous fecal discharge) were observed.

A pulse could not be detected in the femoral artery during callicrein administration, either by palpation or by stethoscopic auscultation used in conjunction with a blood pressure cuff. It often became detectable when the blood pressure rose during the secondary compensation periods, and it usually faded again as the pressure fell. Venous pressures were not measured directly, but it was noted that the superficial veins were collapsed, and it was difficult to obtain blood samples by venipuncture.

Pulse pressure. Comparative measurements of the pulse pressure were made in some of the experiments by tracings from a membrane manometer attached to the carotid cannula. When the infusion of callicrein was first started the pulse pressure increased markedly, as expected from its characteristic action. However, it soon returned to the starting level even though the infusion was continued. Thereafter, changes in the pulse pressure were more or less parallel to the changes in mean arterial pressure; it gradually decreased as the blood pressure fell.

Heart rate. The administration of callicrein to anesthetized or unanesthetized dogs was accompanied by a marked increase in the pulse rate (5), as determined by stethoscopic auscultation of the chest. For example, in one experiment the rate increased from the starting value of 125 beats per minute to 225 during the callicrein administration and then fell to 140 during the next two hours; a gradual increase to 240 preceded death in another two hours. Other dogs showed similar trends, though the exact figures varied. The pulse rate did not always decrease appreciably after it had been speeded up by the callicrein, and these dogs showed progressive increases to 200 to 240 beats per minute until death supervened.

Rectal temperature. Measurements of the rectal temperature by thermometer showed differences between anesthetized and unanesthetized dogs. The rectal temperature did not change appreciably after callicrein administration to anesthetized dogs; in some it fell slowly from 38.3° to 37.8° or 38° C. In unanesthetized dogs, callicrein administration caused the rectal temperature to rise 1 to 2.5° from a starting value of about 39° C. (46). The increase started soon after callicrein administration and was progressive until death.

Cardiac output and oxygen consumption. The cardiac output was determined in anesthetized dogs from the oxygen consumption, as measured by a Benedict-Roth respirometer attached to a tracheal cannula, and arterial-mixed venous

blood oxygen differences determined by Van Slyke analysis (56). Arterial blood samples were taken from the femoral artery, and mixed venous blood samples were taken from a catheter inserted into the right heart through the jugular vein; all blood samples were collected under oil and analyzed immediately.

Figure 2 shows the rapid and very marked decrease in cardiac output that occurred when callicrein was administered. A measurement 1 hour after the callicrein injection usually gave values around 400 cc. per minute. The cardiac output remained at a low level until death, although a slight secondary improvement was observed about the third hour; this probably corresponded to the temporary improvement in well-being noted in the unanesthetized dogs. Blood pressure was not a reliable criterion of the cardiac output when the pressure was high, but a small cardiac output was always found when the blood pressure was very low. Inadequate cardiac output seemed to be the fundamental factor responsible for the development of progressive anoxia and ultimate death.

The oxygen consumption (fig. 2) was also decreased by callicrein; the changes were roughly parallel to the cardiac output, but proportionally less marked and somewhat more erratic. A change in oxygen consumption is usually considered to be a reflection of a change in the metabolic rate; this is based upon the assumptions that the respiratory quotient does not change appreciably and that the oxygen delivered to the tissues is adequate to supply the metabolic needs. The decreased oxygen consumption in shock may be another indication of the inadequate cardiac output rather than a decreased metabolism by the tissues. The accumulation of fixed acids in the blood during shock lends support to this interpretation by indicating that a portion of the metabolism has become anaerobic.

Post Mortem Findings. Autopsy of the dogs after callicrein death showed several gross changes that appeared to be the direct or indirect result of callicrein action. The mesenteric blood vessels were engorged with dark blood and stood out prominently. The peritoneal cavity was very wet if any large amount of saline had been given to the dog. The spleen was contracted and hard. The intestinal veins were hyperemic (22), and infiltration of blood into the lumen of the intestine was visible especially in the duodenum. The stomach was often bloated with gas. Edema of the lungs was observed in only one dog that survived 48 hours before death.

Changes in Blood Constituents. Red cell concentration. One of the first changes observed in the blood after callicrein administration was a marked hemoconcentration. This was observed by *a*, direct measurement by hematocrit; *b*, red blood cell count, or *c*, determination of the hemoglobin concentration of the blood. Typical results obtained by direct measurement are illustrated in figure 3 for normal and splenectomized dogs. The major part of the hematocrit change occurred within one hour of the start of callicrein administration, though it often continued to increase for many hours. Hematocrits changed 10 to 15 points in the normal unanesthetized dog given callicrein, and 10

to 20 points in the anesthetized dog; the nembutal anesthesia alone caused a fall of 3 to 5 points in the hematocrit before the callicrein was administered. A large part of this hemoconcentration was due to contraction of the spleen, since the hematocrit values in splenectomized animals increased only 1 to 8 points. Some cells were also contributed by hematopoietic sites, since nucleated red cells appeared in the blood stream after callicrein. The introduction of new cells into the circulation is a well known response to anoxia. Plasma loss was a third factor responsible for the hemoconcentration.

Plasma volume. Plasma volumes were measured by the Evans Blue dilution method (57); corrections for hemolysis were made in reading the dye concentration in the serum samples. Because of the delayed mixing time of the dye with

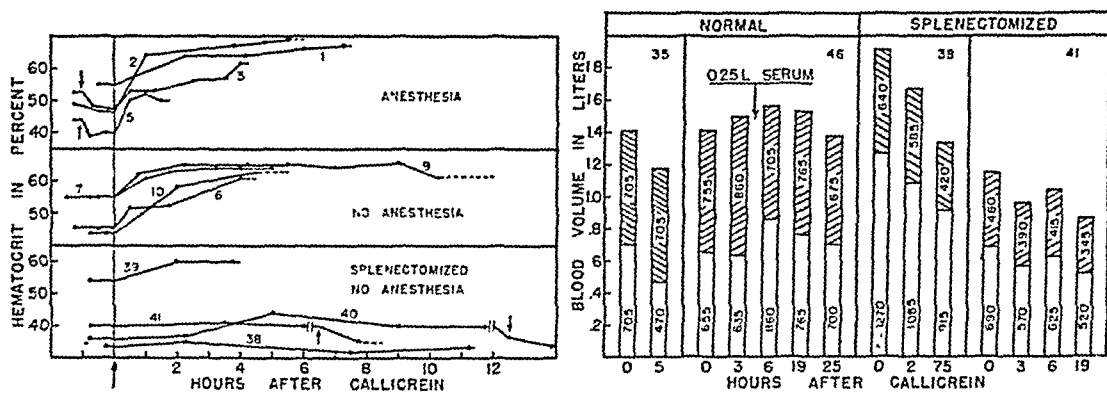


Fig. 3

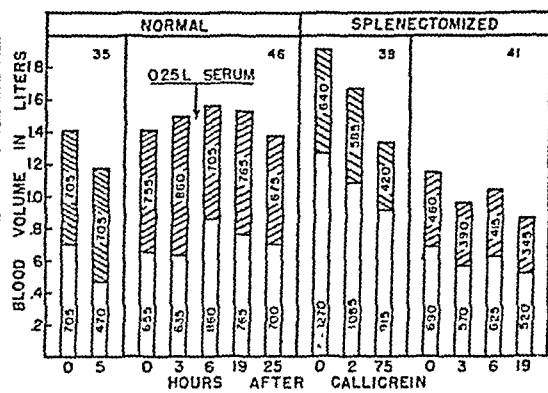


Fig. 4

Fig. 3. Hematocrit changes following callicrein administration.

Callicrein injections were started at the arrow indicating 0 time. Each line has been extended to the time of death. No. 3 and no. 5 received nembutal at the points indicated by the small arrows. No. 1 and no. 2 received nembutal prior to removal of the first blood sample. Breaks in the curves for no. 40 and no. 41 indicate a lapse of 15 and 13.5 hours, respectively, and the small arrows following the breaks indicate the administration of additional callicrein.

Fig. 4. Blood volume change during callicrein shock.

Entire column = total circulating blood volume; shaded area = red blood cells; open area = plasma; figures within respective areas give volumes in ml. 0 time sample = control before callicrein.

the serum after the administration of callicrein, an interval of 15 to 20 minutes was allowed to elapse after the dye injection and before the first blood sample was taken. Seven samples were taken at 4-minute intervals thereafter to obtain the rate of disappearance of the dye; this rate was increased after callicrein administration. Total blood volumes were calculated from the hematocrit and plasma volume data.

The changes in plasma and red cell volumes for several normal and splenectomized dogs are illustrated in figure 4. An apparent loss of plasma is evident in all these experiments. The plasma volume at the time of the last measurement was about 30 per cent less than the original; the amount lost between the time of the last measurement and death is unknown. Dog 46 received a trans-

fusion of 250 cc. dog serum 4 hours after the callicrein, and 2 hours later a measurement showed the presence of 225 cc. more plasma than was present at the preceding measurement. Subsequent determinations showed a continued loss of plasma.

Comparison of the red blood cell volumes shows the difference between normal and splenectomized dogs to be expected from the introduction of cells into the circulating volume by contraction of the spleen. Another effect is evident—i.e., the loss of cells from the circulation, possibly due to entrapment in the capillaries. This latter effect is most clearly seen in the splenectomized dogs where losses of 30 per cent of the original cells occurred. Loss of both plasma and cells contributed to the steadily decreasing total blood volume in splenectomized dogs.

In the normal dogs, the circulating red cell volume was affected by the two opposing forces: *a*, an increase by contraction of the spleen, and *b*, a decrease by loss of cells. Depending primarily upon the time of measurement, there was either an increase or a decrease in the red cell volume. In most of the normal dogs the red cell volume first increased as the spleen contracted, and then gradually decreased as cells were lost from circulation. The total circulating blood volume varied similarly; loss of plasma did not prevent some initial increase in volume, but did contribute to the later decrease.

There is no assurance that this loss of plasma actually represents a leakage from the blood stream. This method of determining plasma volume is open to serious error when applied in shock unless the delayed mixing time is recognized and allowed for in the determination. The mixing time is not clearly demarked in shock as it is in the normal animal, and such errors in measurement give false low values for the plasma volume. Moreover, the method determines only circulating plasma, and any plasma immobilized by stagnation would not be measured. A tremendous decrease in the rate of circulation through certain capillaries would be tantamount to stagnation as far as these measurements are concerned. In view of the apparent loss of red cells from the circulation, this latter possibility is not to be ignored.

Blood oxygenation. The degree of saturation of the blood with oxygen was calculated from the oxygen content (Van Slyke analysis) and the oxygen capacity calculated from the hemoglobin concentration (determined as alkaline hematin) (58). The latter value was occasionally checked by direct Van Slyke analysis of a sample saturated with oxygen.

Arterial blood. In two experiments, the oxygen saturation of the carotid or femoral arterial blood was determined throughout the experiment until death (6-9 hrs.). In each experiment, the hematocrit and oxygen capacity of the blood increased appreciably after the callicrein, but the percentage saturation of the arterial blood did not change although the saturation of the mixed venous blood decreased.

Venous blood. The degree of oxygenation of the venous blood appeared to be the best single criterion for estimating the condition of the animal, since it reflected the failing cardiac output and the approach of respiratory failure. The

cardiac output was actually decreased more than was indicated by the venous oxygen saturation, since the hematocrit increased and the oxygen consumption decreased after callicrein.

Figure 5 shows the changes in venous oxygen content, capacity and percentage saturation for two typical callicrein experiments. The increased hematocrit is reflected in the increased oxygen capacity. Those dogs that died within 3 to 7 hours showed a sharp decrease in the percentage saturation 1 hour after the callicrein and then a more gradual decrease until death. Dogs that survived for longer periods of time showed a secondary rise in percentage saturation before the terminal decline. The venous oxygen saturation was always

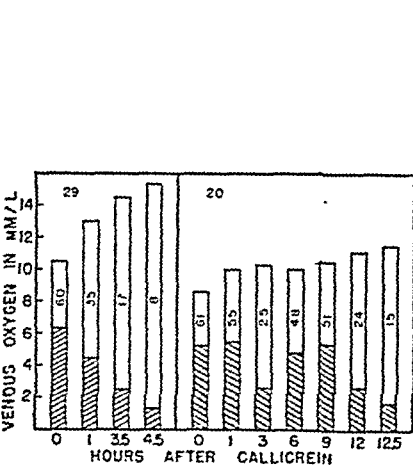


Fig. 5

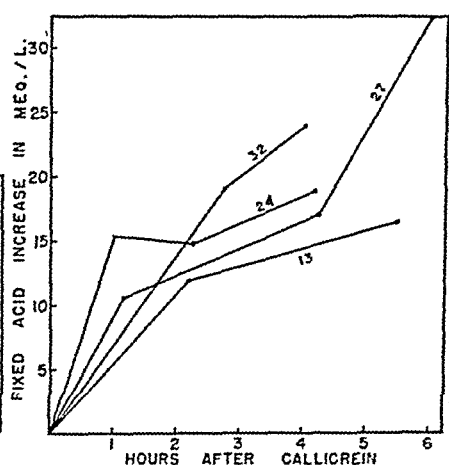


Fig. 6

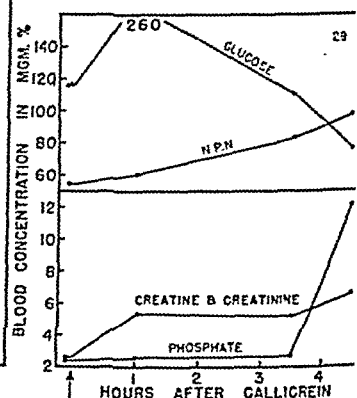


Fig. 7

Fig. 5. Changes in oxygenation of the venous blood during callicrein shock.

Shaded area = oxygen content; entire column = oxygen capacity; figure within column is percentage saturation; 0 time sample = control before callicrein. Last samples were premortal.

Fig. 6. The increase of fixed acids in the blood during callicrein shock. Details of the experiments are given in table 1.

Fig. 7. Changes in some blood constituents after callicrein.

No. 29—Callicrein was given at the arrow. The last blood sample at 4½ hours was premortal. Glucose, N.P.N. and creatine were determined on the separated serum; inorganic phosphate was determined on whole blood.

very low at the time of death, and there could be little doubt that the respiratory failure, which was the immediate cause of death, was due to anoxia. Although some of the dogs showed terminal signs when the venous saturation was around 15 per cent, most of them did not fail until the venous saturation fell to less than 6 per cent.

Acid-base balance. A very intense acidosis due to fixed acid excess usually appeared during the development of callicrein shock (48). In some cases this was severe enough to be incompatible with life; in others the acidosis was a complicating factor. The acidosis appeared to be the result of the anoxia that developed from the inadequate cardiac output. A few measurements of blood lactic acid indicated that more than half of the fixed acid entering the blood

stream was lactate. Normal liver function would be essential for rapid removal of this acid.

Whole blood CO_2 was determined by the usual Van Slyke analysis (56). Blood samples were withdrawn under oil from the jugular vein or from the vena cava via a jugular cannula and analyzed immediately. pH was determined by a Claff modification of the MacInnes-Dole glass electrode (59) except that in experiment 13, it was measured colorimetrically; the values were corrected to body temperature (60). Data from four experiments are recorded in table 1, and from these data the increases in fixed acid entering the blood during callicrein shock were calculated and plotted in figure 6.

TABLE 1
Acidosis in callicrein shock

EXP. NO.	HOURS AFTER CALICREIN	pH	HEMATOCRIT	OXYGEN CONTENT	OXYGEN CAPACITY	WHOLE BLOOD TOTAL CO_2
			<i>per cent</i>	<i>mM/L</i>	<i>mM/L</i>	<i>mM/L</i>
27	0	7.45	47	7.28	9.58	20.9
	$1\frac{1}{6}$	7.35	60	3.95	11.6	16.2
	$4\frac{1}{4}$	7.21	61	7.70	12.2	12.8
	6	6.91	68	0.75	13.2	16.2
32	0	7.34	52	6.06	10.7	20.5
	$2\frac{3}{4}$	7.09	74	2.48	14.6	15.1
	4	7.01	77	0.88	15.2	15.6
24	0	7.30	50	5.24	9.7	24.8
	1	7.12	69	5.14	13.2	16.5
	$2\frac{1}{4}$	7.14	67	3.55	13.4	17.7
	$4\frac{1}{3}$	7.05	71	1.6	13.7	18.7
13	0	7.42	60	9.72	12.5	17.0
	$2\frac{1}{3}$	7.29	74	7.52	15.3	13.0
	$5\frac{1}{3}$	7.23	74	4.01	15.4	13.6

The last sample was premortal in nos. 27, 32 and 24. Dog 13 died one hour after the last sample.

Calculation of fixed acid change. The degree of metabolic acidosis in terms of millimols of fixed acid per liter of blood has been calculated from the analyses of the blood before and at various intervals after callicrein administration. The term "fixed acid change" is retained, although actually the calculation used gives one the change in total available base and includes not only the increase in circulating fixed acids, such as lactic acid, but any decrease in fixed base which may have occurred.

The calculations used are listed below:

(1) The whole blood bicarbonate of each sample of blood was calculated from the pH and $(\text{CO}_2)_b$ using the Henderson-Hasselbalch equation and the appropriate pK.

- (2) The "fixed acid change" in milliequivalents per liter

$$\Delta A = (\text{HCO}_3)_{b1} - (\text{HCO}_3)_{bx}^{\text{corr}} \text{ where}$$

$$\Delta A = \text{"fixed acid change"}$$

$(\text{HCO}_3)_{b1}$ = blood bicarbonate of initial sample, and

$(\text{HCO}_3)_{bx}^{\text{corr}}$ = blood bicarbonate of subsequent sample corrected for pH change, hematocrit change and degree of oxygenation change, according to the equation

$$(\text{HCO}_3)_{bx}^{\text{corr}} = (\text{HCO}_3)_{bx} + \Delta(\text{HCO}_3)_b^{\text{Ht}} - \Delta(\text{HCO}_3)_b^{\text{pH}} - \Delta(\text{HCO}_3)_b^{\text{O}_2}$$

- (3) The blood bicarbonate, $(\text{HCO}_3)_{bx}$, of each subsequent sample was corrected to what it would have been if the blood had had a hematocrit value equal to that of the initial sample.

The equation used for this calculation was:

$$\Delta(\text{HCO}_3)_b^{\text{Ht}} = (\text{HCO}_3)_{bx} \left(\frac{V_{cl}}{V_{cx}} - 1 \right) + (\text{HCO}_3)_{sx} \left(1 - \frac{V_{cl}}{V_{cx}} \right)$$

where

$\Delta(\text{HCO}_3)_b^{\text{Ht}}$ = correction to be added to $(\text{HCO}_3)_{bx}$ as determined, to allow for change in hematocrit.

$(\text{HCO}_3)_{bx}$ = blood bicarbonate obtained from CO_2 analyses and pH.

$(\text{HCO}_3)_{sx}$ = serum bicarbonate obtained from whole blood CO_2 , pH, hematocrit, and degree of oxygenation determinations, and the application of the Van Slyke and Sendroy line chart (61).

V_{cx} = the hematocrit of the blood sample to be corrected.

V_{cl} = the hematocrit of the initial blood sample.

- (4) The blood bicarbonate was then corrected to the value it would have had at the pH of the initial sample of blood, by means of the equation (62)

$$\Delta(\text{HCO}_3)_b^{\text{pH}} = [8.2 + 2.3(\text{Hb})_x] \times \Delta\text{pH}, \text{ where}$$

$(\text{HCO}_3)_b^{\text{pH}}$ = correction to be subtracted from $(\text{HCO}_3)_{bx}$ to allow for change in pH.

Hb_x = total hemoglobin concentration in mM/L in sample to be corrected.

ΔpH = difference between initial pH and pH of subsequent sample.

- (5) Finally, the blood bicarbonate was corrected to the value it would have had if the blood had been oxygenated to the same degree as the initial sample, by the equation (63):

$$\Delta(\text{HCO}_3)_b^{\text{O}_2} = 0.7 \times \left[\frac{\text{O}_2\text{Cont}_1}{\text{O}_2\text{Cap}_1} \times \text{O}_2\text{Cap}_x - \text{O}_2\text{Cont}_x \right]$$

where

$\Delta(\text{HCO}_3)_b^{\text{O}_2}$ = correction to be subtracted from $(\text{HCO}_3)_{bx}$ to allow for change in oxygenation of blood.

O_2Cont = oxygen content in mM/L of blood sample to be corrected (x) and initial sample (1).

O_2Cap = oxygen capacity in mM/L of blood sample to be corrected (x) and initial sample (1).

The blood pH usually fell to very low levels during the development of callicrein shock, and many premortal samples were below pH 7. Blood CO_2 also decreased, as shown in table 1, but neither of these changes illustrate the extent of the acidosis as adequately as the fixed acid increase shown in figure 6. From 15 to 30 mEq. of excess acid were present in each liter of blood. In two other experiments, not shown in figure 6, the increase was also about 30 mEq. per liter.

Organic and inorganic constituents. Changes in several organic and inorganic constituents of the blood were determined in a series of 6 dogs. There were no apparent differences in these values between anesthetized and unanesthetized dogs. Glucose (64), non-protein nitrogen (65), creatine plus creatinine (66), and chloride analyses were carried out on tungstic acid filtrates of the serum. Chloride was determined by a potentiometric titration with silver nitrate. Inorganic phosphate (67) was determined in a trichloroacetic acid filtrate of whole blood. Figure 7 shows the changes in these constituents in a typical experiment; these changes do not appear to be fundamental in the development of shock, nor do they bear any consistent relationship to the condition of the animal or the other aspects of the shock syndrome.

Glucose. Within 1 hour of the callicrein administration, the blood glucose was elevated in all dogs; in some it reached values above 200 mgm. per cent. Thereafter it usually fell until death supervened, and when the animal died rapidly the glucose level usually was still elevated; in experiments lasting 6 hours or more the final value was approximately 60 mgm. per cent or less. The initial increase in blood glucose after callicrein was probably due to the glycogenolysis caused by release of adrenaline (31).

N.P.N. The serum N.P.N. was increased by callicrein in a slow gradual manner. The final levels reached depended upon the starting value and the duration of the experiment. Two alternative explanations for the increased N.P.N. are obvious: a, increased tissue destruction, and b, loss of kidney function. The latter possibility is probable because of the apparent cessation of urine flow.

Creatine plus creatinine. The increase in the total creatine plus creatinine observed after callicrein was due in part to creatinine, but the major part of the increase was due to creatine. The increase was usually rather marked within one hour of the callicrein administration, and then remained stationary for several hours or fell slightly. Another rise occurred before death, and the final values were 2 or 3 times higher than the starting level. These changes in creatine and creatinine may reflect an anoxic breakdown of creatine phosphate.

Inorganic phosphate. Inorganic phosphate was increased at the time of death. In some animals, the starting value was doubled; in others, the increase was very much more marked, as shown in figure 7. The phosphate increased gradually throughout the experiment in some dogs; in others, it remained fairly constant until an hour or so before death and then increased sharply.

Serum chloride. The changes in serum chloride after callicrein were irregular and small. It usually rose within 1 hour of the callicrein (2-6 mEq. per liter)

and then fell gradually, so that at the time of death it might be elevated (short survival) or decreased from the starting level.

Blood cell counts. Red and white cell counts were made in the customary manner in a counting chamber; the white cells were differentiated with the aid of Wright's stain. The results obtained in some of the experiments are shown in table 2. The red cells increased after callicrein administration and closely paralleled the change in hematocrit and hemoglobin concentration. The changes were not identical, since after the callicrein there appeared to be a slight increase in the mean cell volume and a slight decrease in the mean corpuscular hemoglobin concentration. Due to these latter effects, the hematocrit calculated from the hemoglobin concentration was several points lower than the observed hematocrit.

Callicrein caused a marked leucopenia followed by a leucocytosis (52)(48). Most of the animals died during the leucopenia and before the leucocytosis could be established, so that the latter changes are best observed in those dogs that survived the callicrein treatment. Nucleated red cells appeared in the blood after callicrein, and with this exception all the other cell types decreased during the leucopenia. The subsequent leucocytosis was made up almost entirely of polymorphonuclear cells, while the other cell types remained below normal. There were no apparent differences in the white cell response between normal and splenectomized dogs or after nembutal anesthesia.

Hemolysis curves. A number of hemolysis curves were run on blood samples removed before and during the development of callicrein shock to see if any evidence could be obtained of damage to the most accessible cellular membrane, i.e., the red blood cell. The hemolysis curves were run in progressive dilutions of buffered saline, and the degree of hemolysis was read in a photoelectric colorimeter by comparison with a completely hemolyzed sample. There was no consistent or appreciable amount of hemolysis *in vivo*, and most of the hemolysis curves run *in vitro* showed no significant change after the callicrein administration. A few showed some slight increase in fragility of doubtful significance.

Blood coagulation. Changes in the clotting time of the blood were not observed regularly, but in many experiments during the later stages of callicrein shock the clotting time was prolonged. In these cases, the blood samples remained fluid for a long time after small clots could be seen adhering to the walls of the tube. One sample, for example, remained fluid for more than 1.5 hours.

Controls With Inactivated Callicrein. The use of impure callicrein in these experiments leaves uncertain the question of whether all the effects observed were due to callicrein or whether some effects were due to accompanying impurities. The main effect—i.e., the production of shock and death, was controlled by the administration of preparations in which 200 units of callicrein were inactivated either by boiling, acidification, or by incubation with callicrein inactivator. None of the procedures were effective in completely abolishing the hypotensive effect of the injected material on the blood pressure, but none of the animals injected with the inactivated preparations developed shock or died.

TABLE 2
Changes in blood cell counts after callicrein administration

EXP.	CELL TYPE	HOURS AFTER CALLICREIN ADMINISTRATION				
		Control 0	1	2½	4½*	
24	Red blood cells	per mm. ³ 8.13	per mm. ³ 10.69	per mm. ³ 10.40	per mm. ³ 12.36	
	White blood cells	7,000	1,950	1,200	3,350	
	Polymorphonuclear	5,360	390	456	2,560	
	Lymphocytes	736	1,238	468	388	
	Monocytes	140	117	12	50	
	Eosinophils	630	39	180	251	
	Basophils	70	0	0	0	
	Nucleated r.b.c.	0	263	246	100	
29	Red blood cells	0 7.50	1 8.74	3½ 10.58	4½* 11.43	
	White blood cells	5,000	3,150	4,200	4,000	
	Polymorphonuclear	3,600	1,640	3,050	3,050	
	Lymphocytes	975	1,136	613	560	
	Monocytes	250	95	21	100	
	Eosinophils	175	63	275	120	
	Basophils	0	16	0	0	
	Nucleated r.b.c.	0	225	210	200	
31	Red blood cells	0 5.56	2½ 6.61	5½ 7.30	8½ 7.01	12 5.05
	White blood cells	6,850	4,350	17,200	22,150	31,800
	Polymorphonuclear	3,630	3,450	16,630	21,500	31,200
	Lymphocytes	1,300	545	344	310	480
	Monocytes	100	44	0	89	0
	Eosinophils	1,920	217	86	89	0
	Basophils	0	0	0	0	0
	Nucleated r.b.c.	0	87	120	135	70
40	Red blood cells	0 5.04	2½ 5.45	5 6.13	9	
	White blood cells	5,800	2,900	7,900	17,600	
	Polymorphonuclear	4,240	2,260	6,900	16,800	
	Lymphocytes	1,340	522	630	352	
	Monocytes	58	14	0	88	
	Eosinophils	174	43	158	176	
	Basophils	0	14	0	0	
	Nucleated r.b.c.	0	334	276	176	

* Premortal samples.

Red cells are given in millions per mm³.

Dogs 31 and 40 survived.

Heat inactivation was carried out in boiling water for 20 to 30 minutes after slightly acidifying the preparation with HCl to Congo red. Upon intravenous administration of the heat treated material to an anesthetized dog (no.11), the

blood pressure slowly fell about 25 mm. Hg and then returned to the starting level within 1 hour. When given to an unanesthetized dog (no. 17), there was vomiting, retching, defecation and an increased hematocrit from 46 to 60, but the venous oxygen saturation remained above 50 per cent throughout and the dog survived without the development of shock (sacrificed 11 days later).

A preparation of 200 units of callicrein that was dialyzed vs. several changes of 0.01 N HCl for 3 days at room temperature and then running H₂O for 3 days, was injected into an anesthetized dog (no. 21). The blood pressure fell during the injection from 120 to 50, but soon returned to 90–110 where it remained for twelve hours before the animal was sacrificed. The hematocrit increased from 38 per cent cells to 60 per cent as a result of the treatment, but shock did not develop. The dialysate recovered from the 0.01 N HCl by neutralization and concentration *in vacuo* had no effect on the blood pressure, pH, or hematocrit, nor did it cause shock.

When 200 units of callicrein were first incubated *in vitro* with a slight excess of the spleen inactivator (25) and then administered intravenously to an anesthetized dog, shock did not develop. In one dog, the blood pressure did not fall more than 10 mm. Hg, while in another it fell slowly from 140 to 80 during the injection and then rapidly returned to 110. Both dogs showed normal sensitivity to 1 unit test doses of callicrein.

The complete interpretation of these experiments with inactivated callicrein must await the collection of more data. Since shock and death did not develop when the callicrein was first incubated with the spleen inactivator, it is probable that the major effects were due to callicrein, and not to impurities since the same impurities were present in the inactivated preparations.

An interesting point is the apparent separation of the lethal effect from the effect on the intestinal tract by treatment with dilute acid or heat. These inactivated preparations were still quite effective in causing vomiting, intestinal contractions, and increased hematocrit, even though they were not lethal. The evidence in the literature implicates callicrein as the indirect cause of the intestinal effects, and these experiments therefore appear to show a separation of callicrein effects.

Changes in surviving dogs. As noted previously, some of the unanesthetized dogs recovered from the effects of the usual dose of 200 units callicrein, and some anesthetized dogs recovered from smaller doses. Actually, the callicrein effects were very slight in those dogs that recovered. The cardiac output was either relatively unaffected, or it rapidly returned to normal levels after an initial depression. Anoxia did not develop, and the amount of fixed acid entering the blood was slight (2–4 mEq. per liter). The initial fall in blood pressure was similar in all dogs, but in those that recovered the pressure returned to levels about 90 mm. Hg and remained there until the experiment was terminated. The rectal temperature was first elevated by the callicrein, but returned to normal within a few hours. The intestinal contractions were usually less intense, and vomiting did not always occur in those dogs that recovered. Hematocrit changes were small (increased 0–5 points). In some dogs that recovered,

the plasma volume was unaffected; in others, it fell 20 to 25 per cent and remained there for at least 24 hours. The factors responsible for the variations in sensitivity to callicrein are unknown.

Attempts to Reverse the Effects of Callicrein. None of the procedures tested succeeded in preventing death after the administration of callicrein.

Plasma. Callicrein shock was "irreversible" in the sense that plasma did not prevent death when relatively large amounts were given within one hour of the callicrein administration; however, the plasma may have been effective in prolonging the life of the animal. Only small and temporary increases in the blood pressure (5-10 mm. Hg) followed administration of the serum or plasma, but without producing any marked improvement in the venous oxygen saturation. The results of an experiment of this type are presented in table 3.

TABLE 3

Example of the ineffectiveness of plasma in callicrein shock

Expt. 43—8.5 kgm. male dog—nembutal anesthesia.

Blood samples from catheter into vena cava.

TREATMENT	HOURS AFTER CALLICREIN	ARTERIAL BLOOD PRESSURE	HEMATOCRIT	VENOUS O ₂ PER CENT SATURATED
		<i>mm. Hg</i>	<i>per cent</i>	
Normal blood sample after anesthesia and cannulation		145	44	74
200 units callicrein during 20 minutes	0			
	1	80	56	48
200 cc. normal dog serum	1			
	2	90	46	49
	6	75	50	41
	9.5	70	50	43
	11.5	65	50	41
	12.5*	45	50	14

* Premortal sample.

Others. Large amounts of intravenous glucose (40 grams) had only a slight effect on the blood pressure, but increased the venous oxygen saturation very markedly—e.g., from 30 per cent saturation to 70 per cent saturation. The effect disappeared within 30 to 45 minutes.

Intravenous saline or sodium bicarbonate had little or no effect on the blood pressure or the condition of the animal. Tyramine, pituitrin and adrenaline were less effective in increasing the blood pressure after callicrein and were of no permanent value in reversing the syndrome. Sodium glyceroylpoly succinate³ was the most effective substance tried in producing a sustained rise in the blood pressure. Under conditions in which plasma had very little effect on the blood pressure, one gram intravenously raised the pressure 35 to 40 mm. Hg.

³ A high molecular-weight polymer of glycerol and succinic acid developed by Prof. H. T. Clarke of Columbia University.

It did not prevent an eventual deterioration of the animal, and it was ineffective during the later stages of callicrein shock.

Detection of a Shock "Toxin" in Blood. The many failures to obtain evidence for the presence of a toxin in the blood of animals in shock has often been interpreted as evidence against the theory that toxic materials are liberated in shock. Several experiments were, therefore, carried out to determine if callicrein could be detected in the blood after a lethal dose had been given intravenously. Transfusion of serum from a dog that had received callicrein into a normal dog, or the replacement of part of the blood of a normal dog with blood from a dog that had received callicrein was without effect on the recipient, even though the donor would have died from the effects of the administered callicrein. An experiment of this type is described below.

A dog weighing 19 kgm. was anesthetized and injected with 200 units callicrein that caused the usual changes in the blood pressure. One-half hour later, 800 cc. blood were collected into citrate from the carotid artery. This blood

TABLE 4

Blood findings in a recipient dog after partial replacement of the normal blood with blood from a dog that had received callicrein

HOURS AFTER BLOOD TRANSFUSION	BLOOD PRESSURE	pH	HEMATOCRIT	VENOUS O ₂ , PER CENT SATURATED
	<i>mm. Hg</i>		<i>per cent</i>	
1½	100	7.18	54	76
3½	130	7.23	55	74
5½	110	7.17	59	88
7	110	7.22	55	76
7½	110	200 units callicrein administered		
10	60	6.92	69	40
10½*	20	6.88	72	4

* Premortal sample.

Expt. 18—22 kgm. female; nembutal anesthesia; blood samples from catheter into vena cava.

was transfused into a second dog that had been prepared by the rapid removal of 600 cc. blood 15 minutes before the transfusion was started. The blood pressure of the recipient dog was 170 at the start of the experiment, 70 after the hemorrhage and 120 at the end of the transfusion. Blood analysis during the next 7 hours after the transfusion (table 4) showed none of the usual callicrein effects. The injection of 200 units callicrein then produced the usual callicrein changes in the blood and death in 3½ hours, and demonstrated that the recipient dog was not unduly resistant to callicrein. It is concluded that if a toxin of the callicrein type were liberated in shock, it could not be detected by this type of experiment.

DISCUSSION. Some of the effects that callicrein produces can be correlated and explained. The effects of vomiting and defecation are likely due to the substance "DK" formed by the reaction of callicrein with a substance in the blood

serum (34, 35); contraction of the spleen is probably due to the same factor. It is possible that many of the other effects observed with callicrein are, in reality, due to this smaller molecule of unknown composition formed secondarily. Adrenaline elicitation would seem to be responsible for some of the secondary blood pressure changes and for the increased blood glucose. The reduction of the cardiac output by callicrein is believed to be the forerunner of other changes, such as increased pulse, failing blood pressure, anoxia, and acidosis. The acidosis would tend to perpetuate and intensify the callicrein effects since it would liberate free callicrein from the bound form in blood.

If callicrein acts through some chemical action of an enzymatic character, the nature of the latter has escaped observation. The blood analyses furnished no obvious clue to any specific callicrein action. *In vitro* tests showed that callicrein was devoid of hyaluronidase activity, since it did not liquefy synovial fluid mucin⁴. Nor did it attack lecithin to form either lysolecithin (in the manner of snake venoms), or phosphocholine (as does the toxin from *C. welchii*).

The general similarity in many respects of callicrein shock to other types of shock is apparent. A detailed comparison is difficult because of the many variables of experimental technique introduced with a variety of shock-producing methods. The duration of the experiment and the amount of blood lost would appear to be important considerations in any such comparison. A difference between callicrein and clinical shock that might prove to be important is the increased rectal temperature following callicrein administrations; this may be a genuine callicrein effect, but a pyrogenic response from the crude preparations used in these studies must remain of doubtful significance until confirmed with pure callicrein.

This work was undertaken in the belief that callicrein might be involved in the production of shock by trauma, etc. The evidence herein presented shows that callicrein will reproduce the many features of the shock syndrome, including decreased cardiac output, increased pulse rate, failing blood pressure, anoxia and acidosis, increased hematocrit, decreased plasma volume, and even the intestinal effects of vomiting, defecation and bloody diarrhea. The available evidence does not permit a conclusion regarding the relationship of callicrein to clinical shock, except that it is of possible etiological significance.

There are undoubtedly many chemical substances, such as histamine, bacterial toxins, etc., that will produce a shock-like syndrome upon administration. In fact, most of the changes observed in shock will result from any procedure that reduces the cardiac output below the minimum required for adequate oxygenation of the tissues; the many varied methods of producing shock appear to have in common this fundamental effect. Callicrein is unique among the many chemical toxins since it is normally present in mammalian blood in relatively large amounts, and the release of only a fraction of this bound form would be required to produce shock and death.

There are theoretically two possible mechanisms whereby the blood callicrein could be activated—i.e., *a*, by proteolytic destruction of the inactivator, or

⁴ Tested by Dr. Marian W. Ropes of Harvard University.

b, by production of an acidosis to shift the callicrein-inactivator equilibrium in favor of the active form. One of the methods for producing shock depends upon tying off the circulation to one or more limbs for a protracted period of time. Conditions which favor the accumulation of large amounts of acid during this anaerobiosis also favor the production of shock. With a return of blood flow to these areas, the severe localized acidosis could liberate free callicrein from the bound form in the blood. The resulting vasodilatation, plasma loss and edema would remain largely localized so long as the acid entering the blood stream could be adequately buffered or metabolized in other parts of the body. However, a severe plasma loss into the injured area would soon lead to a decreased cardiac output and an ensuing generalized anoxia and acidosis. Widespread callicrein effects could be expected whenever the acidosis became generalized as a result of excessive acid production, decreased liver function, or a combination of such factors.

A type of shock in which additional callicrein may be introduced into the blood stream is produced by intestinal trauma. There are large amounts of free callicrein in the intestinal tract, and the absorption of some of this into the blood stream as a result of trauma could readily account for the shock produced by this method.

The normal physiological rôle of callicrein is unknown. The callicrein-inactivator equilibrium could aid in the regulation of the blood supply to anoxic tissues as a result of the concomitant acidosis and liberation of free callicrein. The hypertensive effect of the callicrein inactivator (25) supports other evidence indicating that some free callicrein normally exists in blood (4). Much larger amounts of the inactive complex as well as an excess of the inactivator complete an equilibrium system that is sensitive to changes in pH (8, 10, 26). If this system exists and operates as suggested, then severe acidosis should produce callicrein shock; actually the clinical findings in the acidosis of diabetic coma are strikingly similar to callicrein shock. Any other mechanism for the cause of death in acidosis is not apparent, and it is significant that the pH within the cells is quite resistant to change (68).

SUMMARY

The intravenous administration of 200 units of callicrein (60 mgm.) to dogs caused irreversible shock and death, usually in 4.0 to 7.5 hours. The mean arterial blood pressure fell precipitously during callicrein administration, partially recovered, and later failed gradually until death. The pulse increased to 200-240 beats per minute. The cardiac output was decreased rapidly and to very low values; the oxygen consumption was changed similarly but to a lesser degree.

At autopsy there was a contracted spleen, engorged mesenteric vessels, hyperemic intestinal veins and infiltration of blood into the lumen of the intestines. Callicrein caused a hemoconcentration of 10 to 20 points in normal dogs and only 1 to 8 points in splenectomized dogs. About 30 per cent of both the plasma and red cells were lost from the circulating blood.

The arterial blood remained normally oxygenated after callicrein, but the venous blood became progressively unsaturated until death resulted from respiratory failure. An intense acidosis developed; the blood pH fell below 7, and the fixed acid increased 15 to 30 m. eq. per liter.

The preparation of callicrein used increased the blood glucose, N.P.N., creatine, and inorganic phosphate. A marked initial leucopenia was followed by a leucocytosis.

Callicrein could not be detected in an active form in the blood after the administration of a lethal dose.

REFERENCES

- (1) HARKINS, H. N. *Surgery* 9: 231, 447, 607, 1941.
- (2) WIGGERS, C. J. *Physiol. Rev.* 22: 74, 1942.
- (3) FREY, E. K. AND H. KRAUT. *Ztschr. physiol. Chem.* 157: 32, 1926.
- (4) KRAUT, H., E. K. FREY AND E. BAUER. *Ztschr. physiol. Chem.* 175: 97, 1928.
- (5) FREY, E. K. AND H. KRAUT. *Arch. exper. Path. und Pharmakol.* 133: 1, 1928.
- (6) KRAUT, H., E. K. FREY AND E. WERLE. *Ztschr. physiol. Chem.* 189: 97, 1930.
- (7) FREY, E. K., H. KRAUT AND F. SCHULTZ. *Arch. exper. Path. und Pharmakol.* 158: 334, 1930.
- (8) KRAUT, H., E. K. FREY AND E. WERLE. *Ztschr. physiol. Chem.* 192: 1, 1930.
- (9) KRAUT, H., E. K. FREY, E. BAUER AND F. SCHULTZ. *Ztschr. physiol. Chem.* 205: 99, 1932.
- (10) KRAUT, H., E. K. FREY AND E. WERLE. *Ztschr. physiol. Chem.* 222: 73, 1933.
- (11) KRAUT, H., E. K. FREY, E. WERLE AND F. SCHULTZ. *Ztschr. physiol. Chem.* 230: 259, 1934.
- (12) WEBSE, H. *Arch. exper. Path. und Pharmakol.* 173: 36, 1933.
- (13) WERLE, E. AND H. KORSTEN. *Ztschr. Ges. Exper. Med.* 103: 153, 1938.
- (14) ELLIOT, A. H. AND F. R. NUZUM. *Endocrinology* 18: 462, 1934.
- (15) GLEY, P. AND N. KISTHINIOS. *Presse Med.* 37: 1279, 1929; 38: 502, 1930.
- (16) FREY, E. K. AND E. WERLE. *Klin. Wehnschr.* 12: 600, 1933.
- (17) WERLE, E. AND P. VON RODEN. *Biochem. Ztschr.* 286: 213, 1936; 301: 328, 1939.
- (18) UNGAR, G. AND J. L. PARROT. *Compt. Rend. Soc. biol.* 122: 1052, 1936.
- (19) KORÁNYI, A., T. SZENES AND B. E. HATZ. *Presse Med.* 45: 779, 1937. *Deutsch. Med. Wehnschr.* 63: 55, 1937.
- (20) WERLE, E. *Biochem. Ztschr.* 269: 415, 1934.
- (21) WERLE, E. *Biochem. Ztschr.* 290: 129, 1937.
- (22) WERLE, E. AND P. ECKEY. *Biochem. Ztschr.* 269: 435, 1934.
- (23) WERLE, E. AND K. URHAHN. *Biochem. Ztschr.* 304: 387, 1940.
- (24) WERLE, E. *Biochem. Ztschr.* 287: 235, 1936.
- (25) GARDNER, L. I., W. W. WESTERFELD AND J. R. WEISIGER. *This Journal* 142: 541, 1944.
- (26) WERLE, E. *Biochem. Ztschr.* 273: 291, 1934.
- (27) WERLE, E. AND J. DÄUMER. *Biochem. Ztschr.* 304: 377, 1940.
- (28) BISCHOFF, F. AND A. H. ELLIOT. *J. Biol. Chem.* 109: 419, 1935.
- (29) WERLE, E. AND A. MARCUS. *Biochem. Ztschr.* 296: 275, 1938.
- (30) FARBENINDUSTRIE, I. G. (Fr. patent no. 843423). *Chem. Abstracts* 34: 6774, 1940.
- (31) BISCHOFF, F. AND A. H. ELLIOT. *J. Biol. Chem.* 100: xvii, 1933; 117: 7, 1937.
- (32) WERLE, E. AND K. FLOSDORF. *Biochem. Ztschr.* 296: 282, 1938.
- (33) WERLE, E. *Klin. Wehnschr.* 15: 848, 1936.
- (34) WERLE, E., W. GÖTZE AND A. KEPPLER. *Biochem. Ztschr.* 289: 217, 1937.
- (35) WERLE, E. AND M. GRUNZ. *Biochem. Ztschr.* 301: 429, 1939.
- (36) SCHRETZENMAYR, A. *Arch. exper. Path. und Pharmakol.* 176: 160, 1934.
- (37) FELIX, J. *Acta med. scand.* 83: 328, 1934.

- (38) LEHMAN, A. J. AND W. VAN WINKLE, JR. *Arch. Internat. Pharmacodyn.* 59: 75, 133, 1938.
- (39) ZIFF, K. AND W. GIESE. *Arch. exper. Path. und Pharmacol.* 171: 111, 1933.
- (40) WIETHAUP, H. *Arch. exper. Path. und Pharmacol.* 168: 554, 1932.
- (41) WERLE, E. *Klin. Wehnschr.* 15: 164, 1936.
- (42) WERLE, E. AND J. HÜRTER. *Biochem. Ztschr.* 285: 175, 1936.
- (43) FREY, E. K., E. WERLE AND E. SACKERS. *Ztschr. Ges. Exper. Med.* 96: 404, 1935.
- (44) RDEKE, T. AND E. WERLE. *Ztschr. Ges. Exper. Med.* 96: 398, 1935.
- (45) NEFFLEN, R. W. AND A. SZAKÁLL. *Biochem. Ztschr.* 269: 80, 1934.
- (46) SZAKÁLL, A. *Arch. exper. Path. und Pharmacol.* 166: 301, 1932.
- (47) GROSS, F., K. MATTHES AND H. GOPPERT. *Klin. Wehnschr.* 19: 73, 1940.
- (48) CREMER, K. *Ztschr. Ges. Exper. Med.* 97: 703, 1936.
- (49) WERLE, E. AND J. LENTZEN. *Arch. exper. Path. und Pharmacol.* 190: 328, 1938.
- (50) ELLIOT, A. H. AND F. R. NUZUM. *J. Pharmacol. and Exper. Therap.* 43: 463, 1931.
- (51) SIVÓ, R. AND E. V. DOBOZY. *Klin. Wehnschr.* 13: 1602, 1934.
- (52) SZAKÁLL, A. *Biochem. Ztschr.* 269: 92, 1934.
- (53) ROCHA E SILVA, M. *Nature* 145: 591, 1940.
- (54) FRENKEL, H. *Klin. Wehnschr.* 13: 1749, 1934.
- (55) CHRISTENSEN, J. F. *J. Path. and Bact.* 48: 287, 1939.
- (56) VAN SLYKE, D. D. AND J. M. NEILL. *J. Biol. Chem.* 61: 523, 1924.
- (57) GIBSON, J. G., 2D AND K. A. EVELYN. *J. Clin. Investigation* 17: 153, 1938.
- (58) EVELYN, K. A. *J. Biol. Chem.* 115: 63, 1936.
- (59) CLAFF, C. L. *Science* 94: 285, 1941.
- (60) SKOTNICKY, J. *Ztschr. physik. Chem.* A191: 180, 1942.
- (61) VAN SLYKE, D. D. AND J. SENDROY, JR. *J. Biol. Chem.* 79: 781, 1928.
- (62) PETERS, J. P. AND D. D. VAN SLYKE. *Quantitative clinical chemistry. Vol. I., Interpretations*, p. 912, 1931.
- (63) HENDERSON, L. J. *Blood.* New Haven, 1928.
- (64) FOLIN, O. *J. Biol. Chem.* 82: 83, 1929.
- (65) WONG, S. Y. *J. Biol. Chem.* 55: 431, 1923.
- (66) FOLIN, O. *J. Biol. Chem.* 17: 463, 1914.
- (67) FISKE, C. H. AND Y. SUBBAROW. *J. Biol. Chem.* 66: 375, 1925.
- (68) WALLACE, W. M. AND A. B. HASTINGS. *J. Biol. Chem.* 144: 637, 1942.

CALLICREIN INACTIVATORS¹

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At least two different substances occur in mammalian tissues that have the common property of destroying the hypotensive effect of callicrein. One of these occurs in the blood plasma (1, 2), while the other is present in lymph nodes (3), cattle spleen (3) and muscle (4). These two callicrein inactivators have quite different physical and chemical properties; the serum inactivator is readily destroyed by treatment with acid, alcohol or by heating to 58° for 45 minutes (1, 2), while the glandular inactivator is unaffected by such treatment (2). Papain digestion destroys the serum inactivator but not the glandular product (2); tryptic digestion destroys both (3). The two substances also differ in their ability to inactivate dog callicrein (5), and treatment with acetone splits only the inactive complex obtained with the serum inactivator (6).

The presence of either one of these callicrein inactivators can be demonstrated by incubating a buffered callicrein solution of known potency with the inactivator and then testing for the usual hypotensive effect of the callicrein upon intravenous injection. One unit of inactivator has been defined as the amount which will inactivate one unit of callicrein in one hour of incubation at 37° in bicarbonate buffer, pH 8 (3). Control of the pH is most important since the degree of inactivation for any given amount of callicrein and inactivator varies directly with increasing pH between 5 and the optimum. The optimum pH is 7.5 for reaction with the serum inactivator (2) and 8-9 for the glandular product (3); neither has any activity below 4 or 5 or above pH 11 (2, 3). The inactivation appears to be due to the formation of a complex between the callicrein anion (isoelectric point of 4.2) and the inactivator cation (isoelectric point of 9-10) (7). The reaction is reversible, and free callicrein can be regenerated by acidification of the complex obtained with either inactivator (3, 4, 6).

A method for the preparation of the inactivator from beef spleen or lymph nodes is described in this report. Various fractionation procedures were followed by assays for the inactivator in phosphate buffer, pH 7.4, and suitable preparations were so obtained for study in intact animals (8). The serum inactivator was also studied and found to be associated with the gamma-globulin fraction.

EXPERIMENTAL. *Spleen inactivator.* Beef spleens were chosen as a readily accessible source of the inactivator, and a satisfactory purification procedure was found to be the following. The spleen was minced and extracted overnight (toluene or xylene preservative) with 8 volumes of dilute acetic acid (3 cc. glacial acetic acid per liter). The filtrate was concentrated in a continuous vacuum still to 1/20 its volume, centrifuged, and the precipitate discarded. Five volumes

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of 95 per cent alcohol were added to precipitate the inactivator; the centrifuged precipitate was redissolved in a small volume of water, and an equal volume of 10 per cent trichloroacetic acid was added. The inactive precipitate was discarded, and the excess trichloroacetic acid was removed from the filtrate by ether extraction; the aqueous phase was dialyzed against running water in a cellophane bag, and the non-dialysable fraction was finally concentrated again *in vacuo*.

Large losses were not encountered in any of the steps, and the final preparation contained approximately 8 units of inactivator for each gram of fresh spleen extracted. Precipitation of the inactivator by the addition of 5 volumes of alcohol was successful when applied to the original extract or concentrate, but led to destruction of the inactivator when carried out after the trichloroacetic acid treatment. When the original extract was cleared with trichloroacetic acid and the filtrate precipitated with tungstic acid, the inactivator was removed from the solution and could be demonstrated in the tungstate precipitate by redissolving the latter in sodium bicarbonate. Sodium tungstate or sodium trichloroacetate did not inactivate callicrein in the test. The inactivator was destroyed by contact with 0.1 N HCl for several days. It was perfectly stable to heating at 58° for 45 minutes, and only a slight loss in activity occurred when boiled for 2 minutes.

An amount of callicrein inactivator required to neutralize the small test dose of callicrein had no effect on the blood pressure of a dog when the inactivator alone was injected intravenously. Large doses were hypertensive. One hundred sixty-five units of inactivator increased the blood pressure 15 mm. Hg for about 1 minute; 420 units gave a 30 mm. rise. The pressor activity remained associated with the callicrein inactivator throughout the limited purification described, and was found in both the lymph and spleen preparations.

Incubation of callicrein with pituitrin or tyramine did not destroy either of the test substances. The effect of such mixtures on the blood pressure was a summation of the hypotensive action of the callicrein and the hypertensive action of pituitrin or tyramine and depended upon the relative amounts of each substance present. The dibasic amino acids and the protamine, salamine, did not inactivate callicrein.

The processing of dog spleen by the procedure developed for beef spleen gave less than 1 unit inactivator per gram of spleen.

Lymph node inactivator. The procedures utilized in preparing the inactivator from beef spleen were also successfully applied to the fractionation of beef lymph nodes. Extraction with dilute acetic acid, precipitation with 5 volumes alcohol, and removal of inactive proteins with trichloroacetic acid gave an extract containing 4 to 8 units of inactivator per gram lymph tissue without appreciable loss in any step. On dialysis of this lymph node extract after ether extraction, however, approximately 50 per cent of the inactivator was recovered in the dialysate, while the remainder was non-diffusible even when dialyzed against running water for 3 days. Very little of the inactivator in spleen extracts passed into the dialysate.

Scrum inactivator. The various protein fractions obtained by Cohn et al. (9)

during the fractionation of normal human blood plasma were tested for the presence of callicrein inactivator. Only fraction 2, which is composed primarily of the plasma gamma-globulins, contained the inactivator. Dried whole plasma also inactivated callicrein at the level of 8 mgm. per unit of callicrein, while fraction 2 comprised 7/65 of the total solids and contained 1 unit of inactivator in 2 to 4 mgm. The leucopenic-leucocytic and possible pyrogenic responses to callicrein (8), and this association of the callicrein inactivator with the antibody fraction of plasma, suggest some relationship between callicrein and infectious processes.

SUMMARY

A partial purification of the hypertensive callicrein inactivator from beef spleen and lymph nodes has been described.

The callicrein inactivator in plasma was found to be associated with the gamma-globulin fraction.

REFERENCES

- (1) KRAUT, H., E. K. FREY AND E. BAUER. *Ztschr. physiol. Chem.* 175: 97, 1928.
- (2) WERLE, E. *Biochem. Ztschr.* 273: 291, 1934.
- (3) KRAUT, H., E. K. FREY AND E. WERLE. *Ztschr. physiol. Chem.* 192: 1, 1930.
- (4) WERLE, E. *Biochem. Ztschr.* 287: 235, 1936.
- (5) WERLE, E. *Biochem. Ztschr.* 269: 415, 1934.
- (6) KRAUT, H., E. K. FREY AND E. WERLE. *Ztschr. physiol. Chem.* 222: 73, 1933.
- (7) WERLE, E. AND J. DÄUMER. *Biochem. Ztschr.* 304: 377, 1940.
- (8) WESTERFELD, W. W., J. R. WEISIGER, B. G. FERRIS, JR. AND A. B. HASTINGS. *This Journal*, 142: 519, 1944.
- (9) COHN, E. J. *et al.* (in press—*J. Clin. Investigation*).

GLYCOLYSIS IN THE PARTS OF THE CENTRAL NERVOUS SYSTEM OF CATS AND DOGS DURING GROWTH

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That the brain can obtain some energy anaerobically was demonstrated in experiments on infant rats by the increase in the lactic acid content of the brain during anoxia (1). With the use of large adult dogs so that both the brain and blood lactic acid could be determined, it was found that in partial anoxia cerebral lactic acid is higher than that of the blood (2). Thus the brain must break down its own glycogen to lactic acid in order to obtain energy. Stone, Marshall and Nims (3) showed that the lactic acid accumulating anaerobically disappears when aerobic conditions are re-established. But even under aerobic conditions the brain persists in forming small amounts of lactic acid (4, 5).

Because the different parts of the brain appear to possess unequal susceptibilities to anoxia (6, 7), a condition in which survival depends on glycolysis, in the present investigation comparative studies were made on the glycolytic ability of the various regions of the central nervous system of dogs and cats during growth.

METHOD. Cats and dogs in several age groups, i.e., newborn to 1 week, 3 to 7 weeks, 3 months, and adults were anesthetized with pentobarbital and the brain and cord were exposed and removed. Various parts of the brain were extirpated: the gray of the cerebral cortex, the caudate nucleus, the thalamus, the corpora quadrigemina, the cerebellum, the medulla, and the cord in the regions of the brachial and the sacral plexus. From each part 100 mgm. of moist tissue were weighed, minced and suspended in Ringer-bicarbonate solution, in an atmosphere of 95 per cent nitrogen and 5 per cent carbon dioxide in the respiratory chamber of a Warburg manometer. The initial readings were made 45 minutes after the time of decapitation. Each step in the process was timed so that all of the tissues were handled uniformly. The readings were taken for one hour. Correspondence was found between this manometric method and the chemical colorimetric of Barker and Summerson (8) when the lactic acid contents of samples of the Ringer-bicarbonate suspension of adult cats' cortex were determined before and after one hour of glycolysis.

RESULTS. The results on the dog (table 1) and cat (table 2) are expressed in terms of CO_2 ; 100 cu. mm. CO_2 are released by the formation of 0.4 mgm. lactic acid which process in turn yields 0.128 calorie anaerobically. These results are analyzed according to Fisher's *t* test (9). The *p* value for each part of the brain is calculated for the difference between the means of the dogs less than 1 week old and 3 to 6 weeks of age, 3 to 6 weeks and 3 months, 3 to 6 weeks and adults, and finally 3 months to adults. In the case of the cats the differences

between the means are calculated for those less than 1 week old and 3 to 7 weeks of age, 3 to 7 weeks and adults. Only p values less than 0.05 are considered significant. Table 3 contains the p values for each part of the brain.

In general the changes in both species follow the same pattern. The rates of glycolysis of the cortex, caudate nucleus, thalamus and corpora quadrigemina are lowest in the newborn and then increase with growth. Other parts of the brain, however, have different patterns during growth. The changes in the

TABLE 1

CO₂ cu. mm. displaced by lactic acid per 100 mgm. moist tissue per hour of the various parts of canine brain

	LESS THAN 1 WK.	3-6 WKS.	APPROX. 3 MOS.	ADULTS
1. Cortex	46 [31-72]* (9)†	117 [92-114] (11)	195 [187-205] (3)	225 [204-252] (5)
2. Caudate nucleus	73 [26-104] (9)	157 [128-220] (13)	256 [248-269] (3)	229 [214-251] (5)
3. Thalamus	104 [74-149] (6)	160 [115-192] (9)	243 [237-253] (3)	193 [156-218] (4)
4. Corpora quadrigemina	116 [96-147] (7)	148 [94-202] (9)	157 [134-191] (4)	85 [70-103] (5)
5. Cerebellum	94 [47-134] (7)	73 [61-98] (9)	124 [113-130] (3)	75 [33-99] (4)
6. Medulla	144 [109-176] (7)	118 [66-182] (9)	61 [38-79] (3)	30 [13-48] (4)
7. Cord	87 [54-113] (9)	38 [14-74] (10)	18 [10-25] (4)	20 [11-27] (5)

* The numbers in brackets are the extreme upper and lower values.

† The values in parentheses are the number of animals used.

cerebellum are not consistent, while the glycolytic rates of the medulla and cord lessen as growth proceeds. In the newborn cat and dog the medulla oblongata glycolyzes faster than any other part at that age. The other portions glycolyze with a decreasing rate in either direction of the neuraxis, whether caudad to the cord or rostrally toward the cerebral hemispheres. In the adult the reverse pattern is seen, lactic acid being formed most rapidly in the caudate nucleus and cortex with decreases in the lower levels of the neuraxis. It is important to

recognize that, in accordance with previous observations (10), the ability of the parts of the brain to glycolyze is highest at some age before maturity is attained. Where this was not illustrated in the table, for example, in the cortex of the dog or the caudate nucleus of the cat, it is possible that additional observations made in animals of intermediate ages might have shown a higher value than in the adult. In general, the glycolytic rates start to increase at an earlier age in the

TABLE 2

CO₂ cu. mm. displaced by lactic acid per 100 mgm. moist tissue per hour of the various parts of feline brain

	LESS THAN 1 WK.	3-7 WKS.	ADULTS
1. Cortex	45 [34-74]* (8)†	188 [147-259] (11)	178 [154-228] (7)
2. Caudate nucleus	85 [64-101] (3)	221 [192-268] (10)	239 [172-295] (7)
3. Thalamus	83 [60-105] (4)	209 [161-259] (10)	167 [129-209] (7)
4. Corpora quadrigemina	105 [72-125] (4)	161 [125-217] (9)	60 [32-98] (6)
5. Cerebellum	95 [77-112] (5)	109 [92-127] (11)	60 [14-91] (6)
6. Medulla	137 [115-150] (4)	103 [70-130] (9)	34 [14-63] (6)
7. Cord	76 [58-117] (5)	27 [19-45] (11)	17 [11-22] (5)

* The numbers in brackets are the extreme upper and lower values.

† The values in parentheses are the number of animals used.

cat than in the dog; the dog brain at 3 months approximates the cat at 3 to 7 weeks.

DISCUSSION. The results obtained in this comparative study of the glycolytic rates of chosen regions of the brain may be compared with the previous ones in which the aerobic metabolism of these same parts of the brain were examined (11). Qualitatively, there are striking similarities, both change in the same direction; quantitatively, however, there are discrepancies because they do not

vary to the same extent. Both the similarities and discrepancies will be considered in the following discussion.

The cerebral oxygen consumption of the newborn dog was lower than that of the adult and the increased metabolism with growth shows the same phyletic phenomenon of rostral progression, as does glycolysis. It would seem that the glycolytic enzymes, like the respiratory (11), augment in concentration with growth, thus accounting for the increasing glycolytic rate observed in the cortex, caudate nucleus, thalamus and corpora quadrigemina. The deposition of white matter, especially in the medulla and cord, produces a different result chiefly because the white matter has a lower metabolic rate than gray (12), explaining in part the decreasing anaerobic metabolism in these two parts.

TABLE 3

P values for the glycolytic activity of the parts of the canine brain according to age

	LESS THAN 1 WK. AND 3-6 WKS.	3-6 WKS. AND 3 MOS.	3-6 WKS. AND ADULTS	3 MOS. AND ADULTS
Cortex.....	<0.01	<0.01	<0.01	>0.01
Caudate nucleus.....	<0.01	<0.01	<0.01	<1
Thalamus.....	<0.01	<0.01	>0.4	>0.05
Corpora quadrigemina.....	<0.02	<0.6	<0.01	<0.01
Cerebellum.....	<0.2	<0.01	>0.5	>0.05
Medulla.....	<0.2	<0.02	<0.01	>0.05
Cord.....	<0.01	<0.05	<0.05	<1

P values for the glycolytic activity of the parts of the feline brain according to age

	LESS THAN 1 AND 3 WKS.	3-7 WKS. AND ADULTS
Cortex.....	<0.01	>0.1
Caudate nucleus.....	<0.01	<0.01
Thalamus.....	<0.01	<0.01
Corpora quadrigemina.....	<0.01	<0.01
Cerebellum.....	<0.01	<0.01
Medulla.....	>0.05	<0.01
Cord.....	<0.01	<0.05

The analysis of the cerebellum is more difficult because the cerebellar parts are of different phyletic ages so that various cerebellar regions may assume characteristic rates as growth proceeds (13).

With glycolysis, as with oxidations, the parts of the brain attain their highest peak some time before adulthood, followed by a recession thereafter. Thus, the developments of glycolysis and oxidations bear a striking resemblance to each other. This resemblance depends upon the mechanisms of aerobic and anaerobic energy production. In accordance with the Embden-Meyerhof scheme (14), the patterns of oxidations and glycolysis are the same starting with glycogen and continuing with the various stages until the formation of pyruvic acid. At this point the two methods of evolution of energy diverge; anaerobi-

cally lactic acid is formed; aerobically, carbon dioxide and water. Since for the most part the paths of glycolysis and oxidations are the same, the lack of exact quantitative relationship may be attributed to the divergence in the terminal portion of the respective paths. These results do not necessarily prove that glycolysis and oxidations take place as part of the same mechanism, for many enzymatic systems may undergo similar changes of concentration during growth. In view of the fact that we have found the medullary metabolic rate highest at birth, both for oxidation and glycolysis, it is interesting that Barron (15) has observed from studies of sheep embryos that in the development of the neuraxis, function begins in the region of the junction of the medulla and cord and is spread in both directions.

Finally the biologic value of cerebral glycolysis should be considered. Though the energy obtained anaerobically is not as great as that arising from anaerobic sources (10), the ability to glycolyze makes it possible for the brain to work beyond its aerobic facilities, a contingency occurring during anoxia (16) and in violent exercise. In anoxia the anaerobically produced energy is more important in the infant (1) than in the adult (2). In exercise the cerebral requirements may be stepped up beyond the aerobic supplies as occurs in convulsions (17). The brain may then call on glycolysis to provide some of the energy to sustain hyperactivity.

SUMMARY

Glycolysis in the parts of the canine and feline central nervous systems has been studied in animals at various stages of their development. In the newborn glycolysis is most rapid in the medulla and is slower in both cephalad and caudad regions. In the adult the highest rates are in the caudate nucleus and cerebral cortex, with lower rates in each succeeding caudad portion of the neuraxis. Glycolysis of the medulla and cord decreases progressively with age. In all cerebral portions studied, lactic acid formation rises to a maximum at some time during development and then recedes in later life. In general, it may be said that the part of the brain exhibiting the highest glycolytic rate advances in a rostral direction as growth proceeds.

REFERENCES

- (1) HIMWICH, H. E., A. O. BERNSTEIN, H. HERRLICH, A. CHESLER AND J. F. FAZEKAS. *This Journal* 135: 387, 1942.
- (2) FAZEKAS, J. F. AND H. E. HIMWICH. *This Journal* 139: 366, 1943.
- (3) STONE, W. E., C. MARSHALL AND L. F. NIMS. *This Journal* 132: 770, 1941.
- (4) NIMS, L. F., E. L. GIBBS AND W. G. LENNOX. *J. Biol. Chem.* 145: 189, 1942.
- (5) HIMWICH, W. A., E. HOMBURGER AND H. E. HIMWICH. *Fed. Proc.* 3: 19, 1944.
- (6) WEINBERGER, L. M., M. H. GIBBON AND J. H. GIBBON, JR. *Arch. Neurol. and Psychiat.* 43: 615, 1940.
- (7) WEINBERGER, L. M., M. H. GIBBON AND J. H. GIBBON, JR. *Arch. Neurol. and Psychiat.* 43: 961, 1940.
- (8) BARKER, S. B. AND W. H. SUMMERSON. *J. Biol. Chem.* 138: 535, 1941.
- (9) FISHER, R. A. *Statistical method for research workers.* Oliver and Boyd, London, 1928.

- (10) CHESLER, A. AND H. E. HIMWICH. This Journal **141**: 513, 1944.
- (11) HIMWICH, H. E. AND J. F. FAZEKAS. This Journal **132**: 454, 1941.
- (12) HOLMES, E. G. Biochem. J. **24**: 914, 1930.
- (13) DOW, R. S. Biol. Rev. **17**: 179, 1942.
- (14) MEYERHOF, O. Biol. Symposia **5**: 141, 1943.
- (15) BARRON, D. H. Biol. Rev. **16**: 1, 1941.
- (16) GURDJIAN, E. S., W. E. STONE AND J. E. WEBSTER. Arch. Neurol. and Psychiat. **51**: 472, 1944.
- (17) STONE, W. E., J. E. WEBSTER AND E. S. GURDJIAN. In press.

ISOTOPIC TRACER STUDIES ON THE MOVEMENT OF WATER AND IONS BETWEEN INTESTINAL LUMEN AND BLOOD¹

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I. STATEMENT OF PROBLEM. The mechanism¹ of movement of materials across biological membranes such as the intestinal epithelium has received much attention but many aspects of the problems involved are still obscure. Studies have been made, for the most part, of net movement of material out of or into the gut. However, the intestinal epithelium is a structure across which materials normally move in two directions (1), and therefore measurements of net transport do not assess the total movement rate in either direction unless the rate in the opposite direction happens to be zero. This is approximately true only for those substances not present in the blood or tissues, that is, for foreign materials. A complication inevitably enters into studies involving abnormal elements, ions, or compounds, namely, that they may alter the behavior of the intestinal epithelium. Furthermore, it is only by inference that any information gained about movement rates of foreign substances can be applied to the problem of movement of the normal components of blood.

The use of isotopes constitutes the only tool available for the study of absolute rates of movement in either direction across the epithelium of the gut, in the cases of substances normally present in the blood of an animal. In this way a variant of the same element, differing appreciably only in nuclear mass but not in chemical properties, can be used as a device to achieve the condition desired, namely, a zero or negligibly small concentration of the material whose movement is to be measured, on one or the other side of the intestinal epithelium.²

In this study simultaneous measurements of movement of water and salt across the intestinal epithelium have been made by labelling the water with deuterium, and sodium or chloride by their radioactive isotopes.

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² The possibility that the radioactivity of the isotopes of elements such as sodium and chloride may influence the behaviour of living structures must not be ignored. However, in the intensities employed in the studies reported here we have been unable to detect an influence, as judged by capacity to do concentration work, which we have found to be a very sensitive indicator of other poisons (2).

In this way data have been obtained which permit a further analysis of the transfer processes occurring across the intestinal epithelium. New information has been obtained which bears directly upon the mechanism of water and salt movement when the net transport is either positive or negative, and when the work done on the material moved is also either positive or negative.

II. EXPERIMENTAL METHODS. 1. *Animal experimental technique.* All observations were made upon dogs under nembutal anesthesia using 30 mgm. per kgm. body weight. The animals were treated with two 2.5 cc. doses of tetrachlorethylene at an interval of one week, at least fourteen days before use. They were deprived of food for twenty-four hours before operation. Two sets of experiments were performed. In the first, ileal segment loops about 12 to 15 cm. in length were prepared after careful washing with isotonic NaCl solution at 37°C., and multiple adjacent loops were studied simultaneously. In the second series the ileal loop lengths were about 20 cm. The placement of various solutions in the lower and upper portions of the ileum was rotated from one experiment to another to avoid a systematic error due to varying behavior at the different levels of the ileum.

After preparation the loops were replaced in the abdomen without torsion and the incision in the latter closed with clamps. After half an hour, each loop was withdrawn separately and aspirated carefully to prove absence of fluid. Then a measured quantity of solution to be studied was inserted and the loop replaced. This procedure was repeated for each loop, from two to four in various experiments. After an interval of time, the same for each loop in a given experiment, the fluid was completely withdrawn by syringe and large bore needle. The procedure employed was to withdraw as much fluid as possible without handling the gut more than holding one end of the loop above the level of the needle inserted at the other. After this, the loop was gently stripped twice toward the needle by an assistant while the operator withdrew the fluid so moved toward the needle. This operation was completed in 10 to 15 seconds. The avoidance of trauma to the gut at all steps is stressed as absolutely essential in studies on intestinal transport. In addition body temperature (rectal) was kept approximately constant by an electric pad and blankets.

2. *Chemical and physical measurements.* Sodium analyses were made by the Barber and Kolthoff (3) method, chloride by the Van Slyke (4) and sulfate by a microgravimetric benzidine method. Deuterium oxide was determined in the larger number of cases by the falling drop method of Keston, Rittenberg and Schoenheimer (5) as modified by Fetcher (6). In the sodium isotope series the floating equilibrium method was employed.

The float density over the range of temperatures and concentrations found was determined empirically using known proportions of D₂O and H₂O. The detailed method of determination was as follows: A filtered 10 cc. sample of gut fluid was boiled gently for 30 sec. after addition of 50–100 mgm. CrO₃, then cooled and filtered into a 100 cc. boiling flask with glass joint. Sufficient KMnO₄ was added to give a distinct purple color and CaO to maintain alkalinity. Porcelain chips were used to prevent bumping. The solution was distilled in vacuo in an all-glass apparatus and the distillate collected. Distil-

lation in the presence of KMnO_4 was repeated three times. In no case was the solution boiled to dryness. All glassware was thoroughly cleaned, washed with triple glass-distilled water and completely dried. The reagents added should be water-free. It is impossible to accomplish this objective perfectly during handling in room air, but the error introduced when every precaution is taken is found to be small. Another unavoidable small error enters in by formation of water in the oxidation of organic matter. The apparent D_2O content of the distillate was measured by determining the temperature of floating equilibrium of a calibrated pyrex glass float. If the floating equilibrium temperatures for the third and fourth distillations deviated by more than 0.05°C ., corresponding to a difference of 0.005 per cent D_2O , the process was repeated until such constancy was attained. The same or better accuracy has been attained with the falling drop method.

The radioactivity of fluid samples was determined on a volume basis, correcting in each case for background count and for decay from zero time, taken as the time of insertion of samples into the gut loops. The values are expressed as RRA, the relative radioactivity on a volume basis corrected for decay. In the chloride experiments a liquid counter built by Wang (7) was employed in order to make measurements rapidly, because of the short half-life of Cl^{38} . The radioactivity in the sodium experiments was measured by the standard technique after drying in aluminum shells. The probable error of the radioactivity measurements is ± 2 per cent.

Radiosodium was prepared by deuteron bombardment of especially pure solid NaOH . It was put in solution and converted to NaCl by titration to neutrality with HCl . Salt concentration was adjusted by dilution to the desired values on the basis of calculations from the titration data. D_2O from 99.5 per cent stock was added to the diluting water in appropriate proportions. Radiochloride was prepared by deuteron bombardment of fused CaCl_2 . After solution in water and acidification with H_2SO_4 the HCl was distilled off and collected. The distillate was neutralized with NaOH and the concentrations adjusted as indicated above.

3. *Calculation procedure.* The primary measurements in this study are of the volumes and D_2O contents of fluid introduced into and removed from gut loops after a period of time, and of the concentrations of sodium and chloride and their radioactive isotopes in the solutions put in and taken out. From these data the absolute directional rates of movement of water and sodium and chloride from gut to blood are calculated by the following equations. The principles underlying their derivation have already been presented (1). For water

$$R_{\text{out}}^w = \frac{(\text{D}_2\text{O})_o \cdot V_o - (\text{D}_2\text{O})_t \cdot V_t}{t \cdot (\text{D}_2\text{O})_m} \dots \dots \dots [1]$$

where (D_2O) represents concentration in per cent, V indicates volume and the subscript g refers to concentrations in gut fluids, o , t , and m refer to zero time, the time of any later observation, and the mean value over the time t .

For chloride

$$R_{\text{out}}^{\text{Cl}} = \frac{[\text{Cl}^*]_o \cdot V_o - [\text{Cl}^*]_t \cdot V_t}{t} \cdot \frac{(\text{SCA})_o}{(\text{SCA})_m} \dots \dots \dots [2]$$

where $[\text{Cl}^*]$ refers to "labelled chloride concentration" and (SCA) the "specific chloride activity" in the solution in question. The latter is a measure of the isotope abundance ratio in relative values. These quantities are calculated as follows:

$$[\text{Cl}^*]_t = \frac{(\text{RRA})_t \cdot [\text{Cl}^-]_o}{(\text{RRA})_o} \dots \dots \dots [3];$$

and

$$(\text{SCA})_t = \frac{[\text{Cl}^-]_o \cdot (\text{RRA})_t}{[\text{Cl}^-]_t \cdot (\text{RRA})_o} \dots \dots \dots [4].$$

Equation [4] is derived from the more fundamental proposition

$$(\text{SCA})_t = \frac{(\text{RRA})_t / [\text{Cl}^-]_t}{(\text{RRA})_o / [\text{Cl}^-]_o} \dots \dots \dots [5],$$

which results from the fact that the specific chloride radioactivity at time t can be related to that at time zero by dividing the radioactivity per unit of chloride concentration at time t by the same ratio at zero time. The resulting value $(\text{SCA})_t$ is not an absolute abundance ratio, but it is such a ratio multiplied by a value, constant for any experiment, which constant is in reality unknown, but is also not necessary to be known for the purposes of these calculations. In these equations $[\text{Cl}^-]_o$ and $[\text{Cl}^-]_t$ are analytical concentrations at times zero and t . $(\text{RRA})_o$ and $(\text{RRA})_t$ are likewise the relative values at the two times of the radioactivity of the solutions in counts per unit time per unit volume, corrected for decay, with $(\text{RRA})_o$ always taken as 100. Corresponding calculations are made for sodium.

The net rate of movement of each material studied is calculated as follows:

$$R_{\text{net}}^{\text{W}} = \frac{V_o - V_t}{t} \dots \dots \dots [6];$$

$$R_{\text{net}}^{\text{Cl}} = \frac{[\text{Cl}^-]_o \cdot V_o - [\text{Cl}^-]_t \cdot V_t}{t} \dots \dots \dots [7];$$

$$R_{\text{net}}^{\text{Na}} = \frac{[\text{Na}^+]_o \cdot V_o - [\text{Na}^+]_t \cdot V_t}{t} \dots \dots \dots [8].$$

It has already been pointed out that for any constituent

$$R_{\text{out}} - R_{\text{into}} = R_{\text{net}} \dots \dots \dots [9].$$

Thus, the several values for R_{into} are obtained by substitution of the two measured values in this equation. The general correspondence between measured and calculated values for R_{out} and R_{into} has been established previously (1).

In the present study the movement of isotope from blood to gut has not been measured and therefore the R_{into} values are not directly measured. Their accuracy depends on the validity of equation [9] and the accuracy of measurement of R_{out} and R_{net} .

III. EXPERIMENTAL RESULTS AND RATE CALCULATIONS. Table 1 presents the observational data from a series of thirty-nine comparable experiments in which salt solutions of concentration from one-third to three times isotonic were introduced into gut loops. In each instance the sub-experiments under a given arabic number were performed on adjacent loops of ileum. Where the initial volumes were not the same for adjacent loops the initial volume inserted was smallest in the case of the hypertonic solution and largest for the hypotonic solution. This was done because the former gained volume and the latter lost volume during the absorption period. It was therefore necessary to rule out gross effects of changing volume on the results. The loops containing the most dilute solution were filled to capacity, and the volume in the others adjusted as indicated.

It is extremely difficult, and perhaps impossible, to obtain exactly comparable areas for absorption in segments of living bowel, because of differences in state of contraction of the musculature. In a sufficient number of experiments differences should, however, result in random variations and should not influence mean values. Furthermore, most of the emphasis in this paper will be placed on the relation between several values measured simultaneously on the same loop of gut, in which relationship the absorbing area is necessarily a constant.

From the raw data in table 1 it is possible to see that the concentration of D_2O in gut loops decreases rapidly, to about half its initial value in ten minutes. The rate of decline is not related in any simple way to the variables studied. Likewise for the radiochloride, although it is possible to see certain trends directly from the data in the table, such as a higher value for $(RRA)_t$ with the more dilute solutions, both the volume and chloride concentration changes need to be taken into account before interpretation is possible.

Table 2 presents the observed data from eighteen observations on D_2O and radiosodium movement and other changes in experiments in which isotonic solutions of $NaCl$ and Na_2SO_4 were placed in adjacent ileal loops. In most cases it will be seen that $(RRA)_t$ is higher in the presence of sulfate than chloride, indicating a smaller loss of radiosodium. But again, further calculations are essential to an analysis of the data.

The above data have been used to calculate the rates of movement of water and ions according to the methods described above. The results appear in tables 3 through 8. It should be noted that the relative magnitudes of R_{out} , R_{into} , and R_{net} for a given loop at a particular time have significance apart from their absolute values, which latter might be influenced by area exposed, while the former (the relative magnitudes) could not be so determined. It will be noted furthermore that there is very little overlap of values for R_{out}^W between tables 3, 4 and 5, for the hypotonic, isotonic, and hypertonic solutions respectively. The standard deviations of the means are small relative to the means

TABLE 1

Experimental observations on volume, chloride, radiochloride and deuterium changes during absorption from ileal segments

EXP. NO.	SALT SOLUTION	TIME	V_o	V_t	$[Cl^-]_o$	$[Cl^-]_t$	RRA_t	D_2O_o	D_2O_t
		min.	cc.	cc.	mE/l.	mE/l.	No./cc./min.	%	%
1 a	53 NaCl	11.5	20.0	8.0	59	81	93.8	1.021	0.388
b	160 NaCl	11.0	20.0	20.8	181	155	79.3	1.046	0.532
c	480 NaCl	10.7	15.0	21.6	478	302	54.3	1.060	0.428
2 a	53 NaCl	10.3	25.0	15.5	52	67	98.5	1.030	0.526
b	160 NaCl	10.2	20.0	24.0	161	130	69.5	1.076	0.453
c	480 NaCl	10.0	15.0	22.2	484	290	58.6	1.085	0.405
d	Iso NaCl-Na ₂ SO ₄	10.1	20.0	19.0	77	69	78.3	1.065	0.643
3 a	53 NaCl	15.0	25.0	15.0	54	65	96.3	1.180	0.623
b	160 NaCl	15.2	20.0	20.5	156	133	81.4	1.126	0.661
c	480 NaCl	14.9	15.0	21.0	482	278	58.7	1.044	0.339
d	Iso NaCl-Na ₂ SO ₄	14.9	20.0	22.5	78	61	46.3	1.237	0.394
4 b	160 NaCl	15.1	20.0	17.5	162	123	76.9	1.184	0.403
b	160 NaCl	15.1	20.0	16.0				1.155	0.456
d	Iso NaCl-Na ₂ SO ₄	15.1	20.0	24.0	88	67	62.8	1.155	0.418
d	Iso NaCl-Na ₂ SO ₄	15.0	20.0	22.0				1.180	0.468
5 b	160 NaCl	16.1	20.0	15.5	156	128	68.6	1.56	0.823
b	160 NaCl	16.2	20.0	16.5				1.62	0.806
d	Iso NaCl-Na ₂ SO ₄	16.1	20.0	14.5	78	40	45.2	1.72	0.739
d	Iso NaCl-Na ₂ SO ₄	16.0	20.0	14.0				1.69	0.832
6 b	160 NaCl	15.0	20.0	13.5	158	136	84.5	1.504	0.715
c	480 NaCl	15.0	15.0	24.0	473	265	52.7	1.406	0.402
d	Iso NaCl-Na ₂ SO ₄	14.9	20.0	18.5	82	51	56.4	1.476	0.643
7 a	53 NaCl	15.6	25.0	6.5	52	98	112.0	1.476	0.423
b	160 NaCl	15.3	20.0	17.0	160	155	55.8	1.473	0.478
d	Iso NaCl-Na ₂ SO ₄	15.1	20.0	21.0	78	85	54.6	1.464	0.422
8 b	160 NaCl	14.9	10.0	8.7	171	134	64.2	1.495	0.398
c	480 NaCl	15.0	6.0	10.5	423	215	44.9	1.403	0.267
d	Iso NaCl-Na ₂ SO ₄	15.3	10.0	7.5	83	45	47.4	1.436	0.340
9 a	53 NaCl	10.1	25.0	12.0				0.739	0.412
b	160 NaCl	9.5	19.5	18.5				0.776	0.502
c	480 NaCl	9.5	10.0	12.5				0.741	0.370
d	Iso NaCl-Na ₂ SO ₄	9.8	18.0	17.0				0.759	0.359
10 b	Iso NaCl	14.6	16.0	14.2				2.32	1.20
11 b ¹	31.6% sea water	15.1	20.0	12.0				2.34	0.88
c ¹	Sea water	14.2	15.0	22.0				2.21	0.87
c	480 NaCl	15.1	15.0	23.5				2.36	0.65
12 b ¹	31.6% sea water	15.4	20.0	16.0				2.34	1.12
c ¹	Sea water	15.7	15.0	19.7				2.21	0.82
c	480 NaCl	17.8	15.0	23.0				2.36	0.47

TABLE 2

Experimental observations on volume, sodium, chloride, sulfate, radiosodium and deuterium changes during absorption from ileal segments

EXPERIMENT		TIME	V _o	V _t	[Na ⁺] _o	[Na ⁺] _t	[Cl ⁻] _o	[Cl ⁻] _t	[SO ₄ ⁻] _o	[SO ₄ ⁻] _t	RRA _t	D ₂ O _o	D ₂ O _t
Expt. no.	Salt solution	min.	cc.	cc.	mE/l.	mE/l.	mE/l.	mE/l.	mE/l.	mE/l.	No./cc./min.	%	%
13 a	160 NaCl	15	25.3	20.6	(162.0)	153.9	(162.0)	134.0	(0.0)		56.3	9.33	4.10
b	256 Na ₂ SO ₄	15	25.0	25.5	(256.0)	237.2	(0.0)	6.4	(256.0)		68.3	8.90	3.14
14 a	NaCl	15	25.1	16.9	160.0	151.4	162.0	140.0	(0.0)		66.6	10.00	4.09
b	Na ₂ SO ₄	15	25.2	22.6	(256.0)	239.2	(0.0)		(256.0)		83.3	9.96	4.63
15 a	NaCl-Na ₂ SO ₄	15	25.0	23.0	(190.0)		(62.0)		(128.0)	138.0		8.50	4.71
b	Na ₂ SO ₄	15	25.0	26.1	(256.0)		(0.0)		(256.0)	252.0		8.47	4.65
16 a	NaCl	20	25.5	19.9	158.7	141.3	158.7	132.3	(0.0)		61.3		
b	Na ₂ SO ₄	20	26.0	25.9	(256.0)	228.0	(0.0)	1.3	(256.0)	247.2	70.5		
17 a	NaCl	20	25.6	24.0	(162.0)	147.8	(162.0)	131.7	(0.0)		72.4		
b	Na ₂ SO ₄	20	26.9	29.3	(256.0)	243.1	(0.0)	2.3	(256.0)	249.2	76.5		
18 a	NaCl	16	25.5	23.0	160.0	154.0	(162.0)	128.0	(0.0)		33.3		
b	Na ₂ SO ₄	16	26.5	35.0	236.0	213.5	(0.0)	19.1	(256.0)		69.2		
19 a	NaCl	30	40.0	34.0	154.0	143.5	154.0	112.0	(0.0)		48.8		
b	Na ₂ SO ₄	30	40.0	44.0	(252.0)	226.5	(0.0)	4.9	252.0	230.0	75.2		
20 a	NaCl	30	20.0	5.3	154.0	139.5	154.0	120.2	(0.0)		44.6		
b	Na ₂ SO ₄	30	20.0	17.0	(243.0)	238.0	(0.0)	0.0	243.0	256.0	80.2		
21 a	NaCl	21	30.0	11.0	(158.0)	139.5	158.0	125.0	(0.0)		49.8		
b	Na ₂ SO ₄	21	15.0	13.0	248.0	235.0	(0.0)	3.0	256.0	260.0	45.7		

Values enclosed in parentheses are concentrations to which solutions were made and are not analytical values.

TABLE 3

Calculations of water and chloride movement between 53mM NaCl and blood

1 EXPT. NO.	2 R _{Cl} out	3 R _W out	4 R _{Cl} into	5 R _W into	6 R _{Cl} net	7 R _W net	8 [Cl ⁻] _{out} ^a	9 [Cl ⁻] _{into} ^a	10 Δ 8-9
	mE/10 min.	cc./10 min.	mE/10 min.	cc./10 min.	mE/10 min.	cc./10 min.	mE/l.	mE/l.	mE/l.
1a	0.76	21.2	0.30	11.0	0.46	9.2	35.8	27.2	8.6
2a	0.56	21.9	0.31	12.7	0.25	9.2	25.6	24.4	1.2
3a	0.42	15.7	0.17	9.1	0.25	6.6	26.7	18.7	8.0
7a	0.74	22.9	0.32	11.1	0.42	10.6	32.3	28.8	3.5
9a		23.1		10.3		12.8			
Mean	0.62	21.0	0.28	10.8	0.35	9.7	30.1	24.8	5.3
σ	0.14	2.4	0.03	1.5	0.07	1.9	4.2	3.7	3.0
σ _m	0.08	1.2	0.02	0.8	0.04	1.0	2.5	2.2	1.8

TABLE 4

Calculations of water and chloride movement between 160 mM NaCl and blood

1 EXPT. NO.	2 R_{out}^{Cl}	3 R_{out}^W	4 R_{into}^{Cl}	5 R_{into}^W	6 R_{net}^{Cl}	7 R_{net}^W	8 $[Cl^-]_{out}^a$	9 $[Cl^-]_{into}^a$	10 Δ_{8-9}
	mE/10 min.	cc./10 min.	mE/10 min.	cc./10 min.	mE/10 min.	cc./10 min.	mE/l.	mE/l.	mE/l.
1b	0.60	11.3	0.24	11.9	0.36	0.6	53.1	20.1	33.0
2b	0.56	13.7	0.36	17.5	0.20	3.8	40.8	20.6	20.2
3b	0.35	6.6	0.09	6.9	0.26	0.3	53.8	13.0	40.1
4b	0.70	13.8	0.02*	12.2	0.72	1.6	50.8	1.6*	52.4
4b		12.9		10.3		2.6			
5b	0.99	9.6	0.28	6.8	0.71	3.2	103.0	41.2	61.8
5b		9.7		7.6		2.1			
6b	0.91	12.2	0.03	7.9	0.88	4.3	74.6	3.8	70.8
7b	1.39	14.3	1.02	12.3	0.37	2.0	97.3	83.0	14.3
8b	0.56	8.1	0.20	7.2	0.36	0.9	69.2	27.8	41.4
9b		9.6		8.5		1.1			
10b		7.7		6.5		1.2			
11b ¹		14.7		9.5		5.2			
12b ¹		10.7		8.1		3.6			
Mean	0.76	11.1	0.28	9.5	0.48	1.7	67.7	26.0	41.8
σ	0.30	2.4	0.30	3.0	0.25	2.1	21.2	24.9	18.1
σ_m	0.12	0.7	0.12	0.8	0.09	0.6	8.2	9.6	6.8

* Negative values for R_{into}^{Cl} or $[Cl^-]_{into}^a$ are impossible except as a result of error in measurement. The values are retained in order to obtain true values for the standard deviation of the mean. The values 0.02 and 1.6 are within the limits of error of measurement from 0.00 in this case and there is no reason to believe that the experimental error in this experiment was larger than in the others.

TABLE 5

Calculations of water and chloride movement between 480 mM NaCl and blood

1 EXPT. NO.	2 R_{out}^{Cl}	3 R_{out}^W	4 R_{into}^{Cl}	5 R_{into}^W	6 R_{net}^{Cl}	7 R_{net}^W	8 $[Cl^-]_{out}^a$	9 $[Cl^-]_{into}^a$	10 Δ_{8-9}
	mE/10 min.	cc./10 min.	mE/10 min.	cc./10 min.	mE/10 min.	cc./10 min.	mE/l.	mE/l.	mE/l.
1c	1.57	8.2	0.98	14.1	0.59	5.9	192.0	69.5	122.5
2c	0.97	9.5	0.14	16.6	0.83	7.1	102.0	7.4	94.6
3c	0.85	8.1	0.08*	12.1	0.93	4.0	105.0	6.6*	111.6
6c	0.75	8.1	0.25	14.1	0.50	6.0	92.6	17.7	74.9
8c	0.51	4.4	0.32	7.4	0.19	3.0	116.0	43.3	72.7
9c		5.0		7.8		2.8			
11c ¹		8.6		14.2		5.6			
11c		6.1		11.0		4.9			
12c ¹		9.4		13.9		4.5			
12c		6.8		9.8		3.0			
Mean	0.93	7.4	0.32	12.1	0.61	4.7	121.5	26.3	95.3
σ	0.36	1.8	0.36	2.9	0.26	1.3	36.1	27.0	19.5
σ_m	0.18	0.6	0.18	1.0	0.13	0.4	18.1	13.5	9.8

* See corresponding footnote to table 4. The same considerations apply to this case.

themselves. Likewise for R_{net}^W there is no overlapping except for three experiments with isotonic solutions in which R_{net}^W was negative. These three values

TABLE 6

Calculations of water and chloride movement between isotonic NaCl- Na_2SO_4 and blood

1 EXPT. NO.	2 $R_{\text{out}}^{\text{Cl}}$	3 R_{out}^W	4 $R_{\text{into}}^{\text{Cl}}$	5 R_{into}^W	6 $R_{\text{net}}^{\text{Cl}}$	7 R_{net}^W	8 $[\text{Cl}^-]_{\text{out}}^a$	9 $[\text{Cl}^-]_{\text{into}}^a$	10 Δ_{8-9}
	<i>mE/10 min.</i>	<i>cc./10 min.</i>	<i>mE/10 min.</i>	<i>cc./10 min.</i>	<i>mE/10 min.</i>	<i>cc./10 min.</i>	<i>mE/l.</i>	<i>mE/l.</i>	<i>mE/l.</i>
2d	0.42	10.4	0.19	9.4	0.23	1.0	40.4	20.2	20.2
3d	0.63	12.5	0.50	14.4	0.13	1.9	50.3	34.7	15.6
4d	0.32	10.9	0.22	13.5	0.10	2.6	29.4	16.3	13.1
4d		10.5		11.9		1.4			
5d	0.68	11.8	0.07	8.5	0.61	3.3	57.7	8.2	49.8
5d		10.8		7.1		3.7			
6d	0.56	11.0	0.09	9.9	0.47	1.1	50.9	9.1	41.8
7d	0.58	14.2	0.73	14.8	0.15*	0.6	40.8	49.3*	8.5*
8d	0.37	8.5	0.05	6.9	0.32	1.6	43.6	7.3	36.3
9d		13.6		12.5		1.1			
Mean	0.51	11.4	0.26	10.9	0.24	0.5	44.7	20.7	24.0
σ	0.14	1.7	0.24	2.7	0.22	2.0	8.7	14.7	18.5
σ_m	0.06	0.6	0.08	0.9	0.09	0.7	3.6	6.1	7.6

* In experiment 7d the discrepancies in columns 6, 9 and 10 from the usual values are so great as to lead the authors to believe that the loop of gut in question was definitely abnormal. However, to avoid the difficulty of setting up criteria for rejecting certain experiments it is included in the means. It is recognized that from some viewpoints the validity of the data would be increased if this experiment were thrown out. No important conclusion is altered by leaving it in, however.

TABLE 7

Calculations of water and sodium movement between 160 mM NaCl and blood

1 EXPT. NO.	2 $R_{\text{out}}^{\text{Na}}$	3 R_{out}^W	4 $R_{\text{into}}^{\text{Na}}$	5 R_{into}^W	6 $R_{\text{net}}^{\text{Na}}$	7 R_{net}^W	8 $[\text{Na}^+]_{\text{out}}^a$	9 $[\text{Na}^+]_{\text{into}}^a$	10 Δ_{8-9}
	<i>mE/10 min.</i>	<i>cc./10 min.</i>	<i>mE/10 min.</i>	<i>cc./10 min.</i>	<i>mE/10 min.</i>	<i>cc./10 min.</i>	<i>mE/l.</i>	<i>mE/l.</i>	<i>mE/l.</i>
13	1.85	15.0	1.23	11.9	0.62	3.1	123.3	103.3	20.0
14	1.73	17.2	0.77	11.7	0.96	5.5	100.5	65.8	34.7
16	1.25		0.63		0.62				
17	0.74		0.44		0.30				
18	2.65		2.31		0.34				
19	1.24		0.48		0.76				
20	1.23		0.45		0.78				
21	2.36		0.83		1.53				
Mean	1.63	16.1	0.89	11.8	0.74	4.3	111.9	84.6	27.4
σ	0.60	1.1	0.59	0.3	0.36	1.2	11.4	18.5	7.2
σ_m	0.22	1.1	0.22	0.3	0.13	1.2	11.4	18.5	7.2

indicate "secretion" of fluid into the gut segment and are unusual, but such behavior has been encountered occasionally with isotonic NaCl solutions in

other experiments. It was at first thought that the radioactivity of the solutions might have caused the unusual response, but a series of control experiments in which solutions identical except for radioactivity were placed in adjacent ileal segments showed no detectable effect of such activity. It was therefore concluded that the condition of the gut at the time of insertion of the sample in the experiments in question was responsible for the result.

In addition to the calculated rates of movement another quantity is shown in columns 8 and 9 of these tables, namely, the apparent concentration of ion in the water moved. This quantity is expressed in $mE/l.$ and can therefore be compared directly with corresponding concentrations in gut loops and blood plasma. Furthermore, the difference in apparent concentrations of ions in the water moving out of and into the gut is presented in column 10. These quantities are believed to be of particular importance because they too are inde-

TABLE 8

Calculations of water and sodium movement between 256 mM Na₂SO₄ and blood

1 EXPT. NO.	2 R _{Na} out	3 R _W out	4 R _{Na} into	5 R _W into	6 R _{Na} net	7 R _W net	8 [Na ⁺] _{out} ^a	9 [Na ⁺] _{into} ^a	10 Δ 8-9
	<i>mE/10 min.</i>	<i>cc./10 min.</i>	<i>mE/10 min.</i>	<i>cc./10 min.</i>	<i>mE/10 min.</i>	<i>cc./10 min.</i>	<i>mE/l.</i>	<i>mE/l.</i>	<i>mE/l.</i>
13b	1.49	15.7	1.27	16.0	0.22	0.3	94.9	79.4	15.5
14b	1.15	12.9	0.45	11.2	0.70	1.7	89.3	40.2	49.1
15b		9.1		9.9		0.8			
16b	1.07		0.68		0.39				
17b	0.65		0.77		0.12				
18b	0.36		1.12		0.76				
19b	0.63		0.59		0.04				
20b	0.56		0.29		0.27				
21b	1.44		1.13		0.31				
Mean	0.92	12.6	0.79	12.4	0.13	0.2	92.1	59.8	32.3
σ	0.38	2.5	0.33	2.4	0.40	1.1	2.8	19.6	16.8
σ_m	0.15	1.8	0.13	1.7	0.15	0.8	2.8	19.6	16.8

pendent of any difference in area or condition of gut epithelium, since they result from simultaneous measurements on a given loop.

IV. DISCUSSION OF RESULTS IN RELATION TO OSMOTIC FORCES. The effects of changes of salt concentration on the movement of water can be ascertained from the data presented above in detailed tabular form. These data are summarized and compared in figure 1. Considering first the net movement, it will be seen that the expected variations in direction of net transport occurred, that is, the net absorption rate has the highest positive value in the case of the hypotonic solution and has a negative value for the strongly hypertonic salt solution. The quantitative differences cannot be interpreted, however, without considering the absolute directional rates of movement.

Looking at the absolute rate of movement of water out of the gut as calculated from D₂O movement, one sees that it too is very much higher in the case of hypotonic than of hypertonic salt solutions. The significance of this observa-

tion, and of the ratios between net and absolute rates, cannot be made clear without considering the fundamental mechanism of osmosis and of diffusion in general.

Normal osmosis is ordinarily considered to be a process in which there is net water transport between two regions or compartments separated by structures permeable to water, in which case the net transport occurs from the region of higher to the one of lower water activity. In such a system we assume that

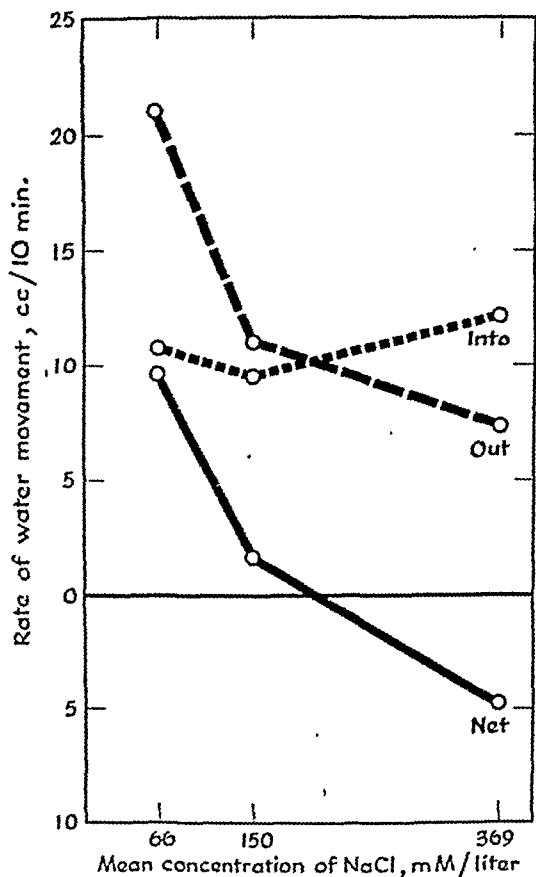


Fig. 1

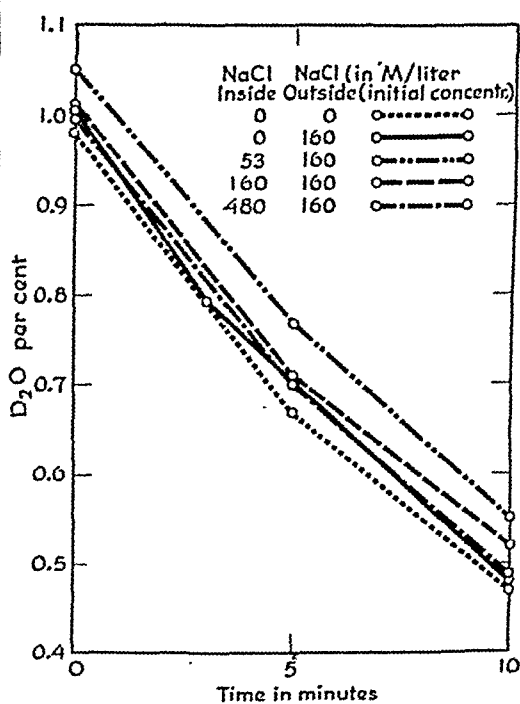


Fig. 2

Fig. 1. Rate of water movement between gut and blood calculated from isotopic tracer data and net volume changes.

Fig. 2. Deuterium oxide movement across the cellophane membrane in relation to salt concentrations.

there must be movement of water by diffusion in both directions across the limiting boundary, the magnitude of diffusion in each direction being determined by the water activities in the respective compartments. Thus the net movement of water in osmosis is the algebraic sum of opposing directional movements. The justification for its consideration as a two-directional process can be found in a treatment by Herzfeld and Smallwood (8).

If one knows the difference in water activity between gut and blood one should be able to calculate the expected ratio between net and absolute direc-

tional movement by diffusion. By comparing such calculated and observed rates it should be possible to ascertain whether normal osmotic forces do or do not account for transport of water across the intestinal epithelium.

A simple treatment³ of the diffusion problem as applied to ordinary inert membranes will make this clear. Fick's law as expressed in terms of concentrations is not applicable. If it is modified by the use of activities it can be written

$$\frac{M}{t} = \frac{KA}{d} \alpha_2 - \frac{KA}{d} \alpha_1 \dots\dots\dots [10],$$

where M/t = net movement of mass per unit time, K the diffusion constant in the membrane or barrier, A the effective area, d the diffusion distance or effective barrier thickness, and α_2 and α_1 active concentrations in compartments 2 and 1 respectively. By definition

$$R_{\text{net}} = \frac{M}{t} \dots\dots\dots [11];$$

$$R_{\text{out}} = \frac{KA}{d} \alpha_2 \dots\dots\dots [12];$$

$$R_{\text{into}} = \frac{KA}{d} \alpha_1 \dots\dots\dots [13].$$

Substituting [11] in [10], and dividing by [12] one obtains

$$R_{\text{net}} = R_{\text{out}} \frac{\alpha_2 - \alpha_1}{\alpha_2} \dots\dots\dots [14];$$

and dividing [12] by [13]

$$\frac{R_{\text{out}}}{R_{\text{into}}} = \frac{\alpha_2}{\alpha_1} \dots\dots\dots [15].$$

From [14] it is evident that when active concentrations are known R_{net} for an osmotic process can be calculated from R_{out} , if it is known, or vice versa. For this purpose it is quite unnecessary to know D , A , or d , so long as the system is considered to behave ideally⁴.

These three values are constants which cannot be determined accurately by

³ It is recognized that equation [10] is not a completely general expression for transport across a membrane, but it is sufficiently adequate for simple osmotic transport to permit its use in the cases considered here.

⁴ The work of Berkeley and Hartley (9) has shown that the net transport rate of water across a semipermeable membrane is governed strictly by Fick's law. They further showed that although the effectiveness of stirring was important in determining water transport rates at high osmotic concentrations, the effect of stirring virtually disappeared at concentrations about 1 molar. At that concentration the instantaneous rates observed with violent stirring and with no stirring were the same within experimental error. Since all the present studies involve much lower concentration differences it may be inferred that the possibly poor stirring in the gut lumen will not seriously influence the results reported.

direct measurements. It is fortunate that for any single experiment their values need not be known in order to answer the questions proposed. These questions are: 1, what net rates of transport of water would be expected from the observed directional movement rates if normal osmosis were the mechanism of movement; 2, what ratio between R_{out}^W and R_{into}^W should exist on the same assumption; and 3, how well do the predicted rates agree with the observed values?

The answers are very clear-cut in their implication. The activity of water can be calculated from the mol fraction and expressed in terms of vapor tension. In such a calculation the fact that deuterium is present almost exclusively as HDO must be taken into account. For purposes of this calculation it is assumed that all the deuterium is in the latter form. The abundance ratio of H_2O and HDO must be known in each case and taken into account because the

TABLE 9
Composition and osmotic properties of plasma and gut solutions

COMPONENT OR VALUE	UNITS	PLASMA	GUT SOLUTION		
			Hypotonic	"Isotonic"	Hypertonic
1. H_2O^*	Molality†	55.508	54.638	54.300	54.348
2. HDO*.....	Molality	0.000	0.822	1.143	1.099
3. Solute*.....	Molality	0.320	0.132	0.300	0.738
4. Total.....	Molality	55.828	55.592	55.743	56.185
5. Ratio: (1)/(4).....		0.9943	0.9829	0.9741	0.9673
6. Ratio: (2)/(4).....		0.0000	0.0148	0.0205	0.0196
7. H_2O V.T. 37.5°C‡.....	Mm. Hg	47.681	47.124	46.701	46.375
8. HDO V.T. 37.5°C§.....	Mm. Hg	0.000	0.665	0.918	0.880
9. Total Water V.T.....	Mm. Hg	47.681	47.789	47.619	47.255

* Mean values over the time of observation.

† Gram mols per 1000 grams water ($H_2O + HDO$).

‡ Vapor tension of H_2O at 37.5°C taken as 47.944 mm. Hg.

§ Vapor tension of HDO at 37.5°C taken as 44.900 mm. Hg. A linear variation for fractional mixtures is assumed.

activities and vapor tensions of the two are different. The calculations of water vapor tensions in the several solutions are shown in table 9. For the purposes of these calculations it is assumed that the salt solutions are ideal. The equivalent osmolality of plasma, 0.160 M NaCl, is derived from vapor tension measurements.

It can be assumed that water activity is proportional to its vapor tension. Since we are interested only in ratios of activities the water vapor tension ratios have been employed directly.

From these data the expected rate ratios can be derived, assuming that the movement from gut to blood occurs under normal osmotic forces. It is apparent from a study of table 10 that osmosis theory as outlined does not account for the quantitative aspects of transport of water between gut and blood. The largest discrepancy between calculation and observation is in the case of net

movements. In each case the observed net movement is more than sixty times what would be expected. It may be noted that the hypotonic solution shows net absorption 200 times greater than prediction from osmotic theory, assuming R_{out}^W to be a correct measure of total directional movement. Moreover, for the "isotonic" solution the observed value is opposite in sign to prediction.

Less assurance can be placed upon the R_{into}^W values because they are not as accurately known, and because smaller proportionate differences between calculated and "observed" values are to be expected, but, especially in the case of the hypotonic solution, the difference of 10 cc. between calculated and "observed" is far too great for errors in measurement to be responsible.

As noted above all the calculations just discussed are of simultaneous movements across the epithelium of a given loop of intestine or group of comparable loops and do not depend upon a knowledge of the absorbing area of the gut. The evidence from such calculations is the most compelling, but since other

TABLE 10

Mean rates of water movement between gut loops and blood compared with predictions from osmotic theory

SOLUTION	1 R_{out}^W OBSERVED	2 R_{net}^W CALCULATED FROM EQUATION [14]	3 R_{net}^W OBSERVED	4 R_{into}^W CALCULATED FROM EQUATION [15]	5 R_{into}^W "OBSERVED"*
Hypotonic.....	21.2	+0.05	+9.2	21.1	11.0
"Isotonic".....	11.1	-0.01	+1.7	11.1	9.5
Hypertonic.....	7.4	-0.07	-4.7	7.5	12.1

* The values in column 5 are derived by substituting from columns 1 and 3 into equation (9). They are really inferred rather than observed figures. They may be in error to the extent that R_{out} and R_{net} measurements are inexact.

data bearing on the same question are available, they should be considered for what they may be worth as corroborative evidence.

It is ordinarily considered to be permissible to use adjacent loops of gut of the same lengths for comparison of absorption under different conditions. If this type of control is adequate the data presented have additional implications. First, it is seen (fig. 1 and table 10) that R_{out}^W does not vary in linear proportion to the water activity in the gut, values for which appear in table 9. If it be assumed that simple diffusion is the mechanism of water movement from gut to blood and one calculates R_{out}^W values expected at various salt concentrations, taking the "isotonic" solutions as the standard of reference one would expect the values for the hypotonic and hypertonic solutions to be 11.12 and 11.03 cc. instead of the observed 21.2 and 7.4 cc., respectively. The predicted difference between the 0.369 M and 0.066 M solutions is 0.1 cc. and the observed is 13.8 cc., or 138 times the predicted value. This difference is relatively very large, and could scarcely be due to observational error.

In contrast to the unpredictably large variation in R_{out}^W for salt solutions of various concentrations, the values for R_{into}^W do not appear to depend upon the

salt concentration in the gut in these experiments to any large extent. This result is in itself not out of harmony with simple osmotic theory but it would likewise be predicted from any theory which depended upon constancy of blood composition and membrane properties. However, the observations are incompatible with any theory which would invoke an effect of salt concentration in the gut upon water movement into it.

A comparison of R_{net}^W values observed at the various salt concentrations leads to significant inferences. If the value for the hypotonic solution is used as a base for calculation, the expected R_{net}^W value for the hypertonic solution should be -35 cc., instead of the observed -4.7 cc., a difference outside any possibility of observational error. The comparatively low observed R_{net}^W for the hypertonic solution is out of harmony with a simple osmotic theory of transport.

The calculation for expected net movement makes no assumptions except that the diffusion law holds and that adjacent loops can appropriately be considered as having identical properties and can therefore be used as simultaneous controls. The validity of the conclusion does not depend, therefore, on the accuracy of measurement of total directional rate of water movement.

However, the comparison of movement rates in adjacent loops filled with different solutions depends for its justification upon the assumption that the permeability of the living epithelium to water is not significantly altered by the solution composition changes. Since the living epithelium appears to make an active contribution to transport it is not possible to consider changes in rate of water movement across it as measures of permeability differences. In some way the active and the passive components of the membrane effect will have to be separated one from another. Since the possible effect of the osmotic pressures of the solutions bathing it on its two sides upon the permeability of a membrane to water is extremely important to the interpretation of the data presented, we have performed experiments on non-living membranes. We have employed for this purpose the cellophane membrane, which, according to Sollner (10), is practically devoid of electrochemical activity and therefore should not introduce complications due to abnormal osmosis.

A cylindrical membrane 15 cm. long was prepared from 28 mm. cellophane tubing. It was filled with 30 ml. of either water or salt solution of composition indicated in figure 2 containing about 1 per cent D_2O , suspended in 600 ml. of external liquid of composition indicated, and stirred with motor driven glass rods and vanes. The decline in concentration of D_2O in the internal liquid was measured over time. The changes in volume of the internal solution over the time of observation were not measured in this series of experiments, but other observations have shown that they would be of the order of 10^{-3} times the volume of the internal solution and are therefore negligible for the purpose at hand.

The results are shown in figure 2, where it can be seen that the rate of loss of D_2O from the internal solution over 10 minutes is not measurably different when the inside and outside liquids are *a*, water; *b*, NaCl solutions of equal concentration, or *c*, NaCl solutions of differing concentrations. The rates are

the same, within the limits of accuracy of measurement, regardless of whether the net osmotic flow is in the same, or in the opposite direction, as the direction of D_2O transport. Thus it is evident that the rate or direction of net osmotic flow does not greatly influence water exchange by diffusion, and that the osmotic pressure of the solution bathing the cellophane membrane does not determine its permeability to water⁵.

These model experiments appear to prove that, for membranes behaving like cellophane, water permeability is not influenced by osmotic pressure changes over the range of physiological importance. A similar study of natural membranes is under way and will be reported later.

One complication in the method employed for measuring total directional movement of water by D_2O tracer measurements must be discussed. When D_2O enriched solutions are placed in a gut loop there is exchange with all of the water in adjacent intestinal epithelium to which there can be access through water-permeable membranes. In other words a part of the D_2O leaving the gut lumen in these studies may not be transferred to the blood at all but be left in the intestinal wall. In this case the calculated R_{out}^W would be fictitiously high. This point is crucial to the validity of the foregoing conclusions. If R_{out}^W is really smaller than the figures given above would indicate, several points are of interest: 1. The discrepancy between calculated and observed values of R_{net}^W would be increased, thus making the classical osmotic theory still more untenable. 2. The differences in R_{out}^W for solutions of various salt concentrations might be partially accounted for by such a phenomenon. Epithelial cells should take up water from hypotonic solutions and should give up water to hypertonic solutions and therefore the entrance of D_2O into cells should be greatest in the first and least in the last case, being intermediate for the isotonic solution. This would agree with the trends observed. Therefore it is evident that some part of the differences in R_{out}^W observed at various salt concentrations may be due to this effect. 3. An attempt has been made to assay the possible error due to the "storage" of D_2O in the epithelium. We have found that 5 to 10 per cent of the D_2O leaving the lumen of the intestine may be in the mucosa after a 10 minute period of absorption from isotonic solutions. Therefore, we estimate the probable error in calculated rates from this source to be not more than that value. A correction of this magnitude would not alter previous conclusions.

Attention is next called to table 6 in which it appears that when mixtures of $NaCl$ and Na_2SO_4 in isotonic solution are placed in ileal segments the value R_{out}^W is almost identical with that found with isotonic $NaCl$ (table 4). The values for R_{into}^W are significantly but not greatly different in the two series. The intimate reason for the latter difference is not obvious, but the difference correlates with the lower value for R_{net}^W in the presence of SO_4 ion in fairly high concentration.

The data in table 2 and the calculations shown in tables 7 and 8 are from

⁵ These measurements are not accurate enough to detect the small differences in total directional movement caused by the changes in water activity due to altered solute concentration.

another set of experiments, in which loop segment lengths and initially inserted volumes were both larger than in the experiments in table 1. Therefore absolute values of rates cannot be compared, but the ratios can be. For isotonic NaCl the ratio R_{out}^W/R_{into}^W equals 1.37, as the average of the two observations made. This compares with 1.17 noted in the larger series in table 4. The difference is not outside the range of variation observed in the larger series and cannot be considered significant. When Na_2SO_4 is considered, the value for R_{out}^W is not significantly different, but the ratio R_{out}^W/R_{into}^W for the two Na_2SO_4 experiments is 1.02. This ratio is also within the spread of observations for isotonic NaCl and cannot be called significantly different. It should be pointed out, however, that the expected ratio should approach 1.00 when R_{net}^W equals zero, as it does frequently with isotonic Na_2SO_4 in ileal loops. A zero value for R_{net}^W with isotonic NaCl is definitely unusual, and when it occurs is believed to result from previous injury to the intestine or to stimulation of the intestinal glands.

The main facts concerning the movement of water may now be re-stated.

1. The total directional movement of water from gut to blood as measured by isotopic tracer methods has been compared with the net transport measured directly. When osmotic gradients exist the net movement is sixty or more times that which would be predicted on the basis of osmotic theory. This result is derived from simultaneous measurements on single loops of gut and does not depend upon any assumptions as to absorbing area, diffusion distance (membrane thickness) or membrane permeability, except that these constants are effectively the same for movement of water by diffusion in the two directions.
2. The total directional movement of water from blood to gut, obtained from substitution of observed values for R_{net}^W and R_{out}^W into the equation $R_{into}^W = R_{out}^W - R_{net}^W$, is found to be at variance with the quantities calculated on the assumption that R_{out}^W represents the directional movement by diffusion. The discrepancy is greatest in the case of hypotonic gut solutions. As in the previous case, this conclusion depends upon simultaneous measurements on the same segments of intestine and therefore could not be vitiated by any plausible observational errors. Any escape from the conclusions resulting would have to come by discovery of flaws in the application of osmotic theory.
3. If it be assumed that equal lengths of adjacent loops may appropriately be compared it appears that the total directional rate of movement out of the gut is about twice as great from 0.066 M NaCl as from 0.150 M or 0.369 M. The observed difference between 0.150 M and 0.066 M is 100 times the predicted.
4. For the total directional rate into the gut, the "observed" values are nearly the same regardless of salt concentration changes in the gut.
5. The net movement between gut and blood does not follow predictions from simple osmotic theory. This last observation is independent of the validity of the isotopic tracer method of measuring directional rates of movement. However, it is not as conclusive as the former because the assumption underlying its interpretation, that the permeability of the gut wall to water is unchanged by alterations in salt concentration, is untested. Therefore the tracer

studies on simultaneous movement must be considered as the major evidence for the conclusions reached.

V. DIFFUSION PROCESSES AND ION TRANSPORT. Due to electrical forces the movement of a single ion species across a barrier cannot be considered without taking into account transport of ions of the opposite sign or exchange with those of the same sign. Consequently, the treatment of chloride ion movement rates cannot disregard the simultaneous diffusion of counter ions and exchange. As far as the first is concerned, it is probably fair to assume as a first approximation that sodium and chloride ions can move simultaneously from gut to blood and in the reverse direction with ease. At any rate they both pass from one to the other readily, as many earlier observations have shown. Therefore it seems justifiable to test whether or not observed movement rates obey the predictions of diffusion theory.

The chloride transport rates can be treated as were the values for water movement. Equations 14 or 15 allow one to determine whether the observed ratios between R_{out}^{Cl} , R_{into}^{Cl} , and R_{net}^{Cl} , agree with calculations from one of those values and the known (measured) concentration gradients. The values for the above rates at the three NaCl concentrations employed are shown graphically in figure 3. The first point to which attention should be called is that R_{net}^{Cl} , at all values of $[Cl^-]_g$ studied, is more than 50 per cent of R_{out}^{Cl} . This is true even at 0.066 M NaCl per liter, where the chloride concentration gradient is negative, the plasma level being about 0.100 M. In this case net movement of chloride by diffusion from gut to blood should be negative. Calculating from the observed R_{out}^{Cl} according to equation 16 the expected value for R_{net}^{Cl} would be -0.21 , in contrast with the observed value $+0.35$ mE per 10 minutes. The observed large positive absorption is out of harmony with the classical diffusion theory of transport.

Likewise, in the case of the isotonic solutions of mixtures of NaCl and Na_2SO_4 , the direction of movement is opposite to diffusion theory prediction. From the concentrations given in table 1 it can be seen that the mean value for $[Cl^-]_g$ over the time of absorption in these experiments, designated as d is 0.070 mE per liter. The mean plasma $[Cl^-]$ is about 0.100 mE per liter. From table 6 it appears that R_{out}^{Cl} is 0.51 mE per 10 minutes. From equation 14 one calculates that R_{net}^{Cl} should be -0.15 , if due to diffusion. The value found, table 6, is $+0.24$ mE per 10 minutes. Again it seems that this net movement depends on other than simple diffusion forces⁶.

⁶ A common suggestion as to the mechanism of establishment of concentration differences across living membranes is that differential permeabilities to various ion species allow the setting up of membrane equilibria of one or another sort. In this way, it is supposed, concentration energies of one component may be utilized to drive other components against concentration gradients so as to perform work on the second component. This mechanism may operate in some instances to produce and maintain concentration gradients. The question in point is as to whether it is of importance in the situation under discussion in this paper.

In any membrane equilibrium the following relation between active concentrations

At the NaCl concentration 0.150M the expected value for R_{net}^{Cl} , calculated from equation 14 and the observed R_{out}^{Cl} , would be 0.25 mE per 10 minutes. The measured value is 0.48 mE per 10 minutes. The difference between the two is greater than the experimental error.

In the case of the hypertonic solution the calculated R_{net}^{Cl} is 0.68 as compared with the observed 0.61 mE per 10 minutes. Here there is no inherent disagreement. However, in view of the discrepancies in sign and in magnitude in the other two cases, it seems necessary to conclude that the agreement in the case of the hypertonic solution is probably fortuitous.

If the chloride movement rates in adjacent loops are compared one finds, as in the case of water movement, that the rates are not in proportion to the concentration differences. For R_{out}^{Cl} the value at 0.066 M NaCl is 0.62 mE per 10 minutes. With 0.369 M NaCl one would expect about 6 times as great an outward movement, or 3.60 mE per 10 minutes. The observed value was 0.93 mE per 10 minutes. For the isotonic solution a comparable discrepancy exists between expected and observed rates. Similar difficulties are encountered if one compares net movement rates under the several concentration conditions. It can be concluded that if adjacent loops can be considered to be comparable, and if the concentration differences do not in themselves cause permeability changes, these data are out of harmony with classical diffusion theory as regards chloride movement.

The movement of chloride from blood to gut is remarkably independent of its concentration in the intestine. The mean values of R_{into}^{Cl} for the hypo-, iso- and hypertonic solutions respectively are 0.28, 0.28 and 0.32 mE per 10 minutes. This finding is in line with the fact that the plasma concentrations remain a constant, but if forces other than concentration gradients are causative for movement, the above finding would indicate that whatever forces move material from blood to gut they are largely independent of uni-univalent salt

must prevail at equilibrium with respect to any and all univalent ions to which the membrane is permeable.

$$[Cation]_1 \times [Anion]_1 = [Cation]_2 \times [Anion]_2 \dots\dots\dots [16]$$

Furthermore, in any such system proceeding toward equilibrium the changes in ion concentration products must be in the direction toward the equilibrium condition. It has previously been pointed out (11) that when uni-univalent salt impoverishment occurs in the intestine the reverse phenomenon is seen.

However, a point which could not be settled previously is now elucidated. The present isotope studies show that the permeability of the gut to sodium and chloride ions is about the same, as judged by the values of R_{out}^{Cl} and R_{out}^{Na} under comparable conditions, as can be seen in tables 4 and 7. Allowance must be made for the somewhat larger absorbing area (loop length) in the sodium experiments. Thus it cannot be assumed that the gut wall is impermeable to either Na^+ or Cl^- . This supposition had, as a matter of fact, been rejected earlier, on the basis of indirect evidence. The isotope studies serve, however, to make the rejection imperative. Equation [16] must, therefore, hold if a membrane equilibrium accounts for the concentration changes. Since the deductions of equation [16] are not verified it must be concluded that absorption of uni-univalent salt counter to concentration gradients is not due to membrane equilibrium effects.

concentration in the intestine, because that concentration was varied over a ninefold range in these studies. This is in remarkable agreement with the findings in connection with water movement from blood to gut, which is also virtually independent of gut fluid salt content and osmotic pressure under the conditions studied⁷.

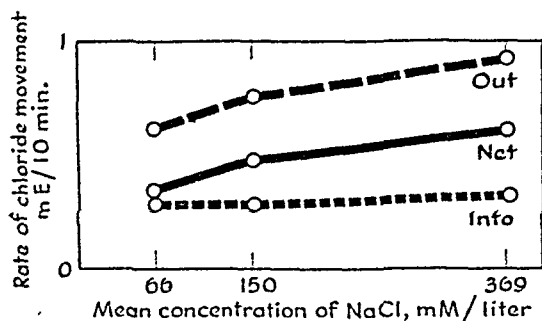


Fig. 3

Fig. 3. Rate of chloride ion movement between gut and blood calculated from isotopic tracer and analytical data.

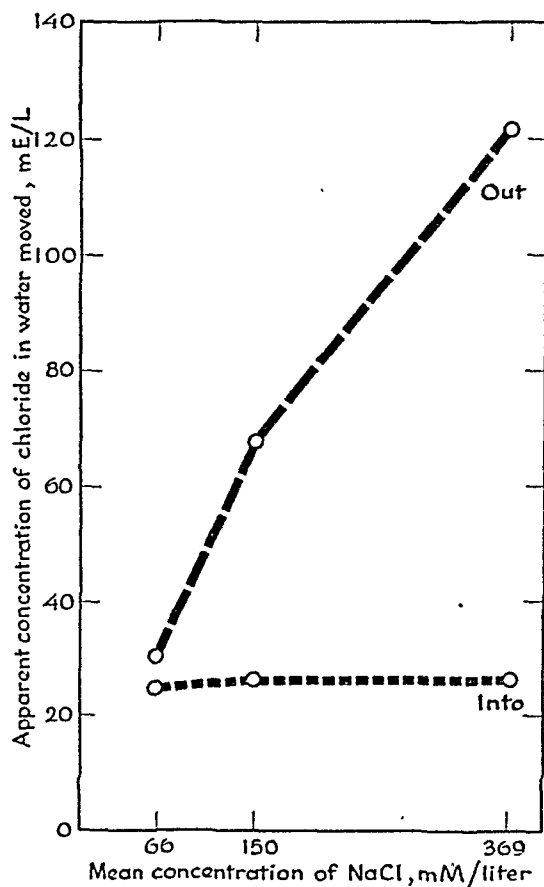


Fig. 4

Fig. 4. Apparent concentration of chloride ion in the water moving between gut and blood in relation to gut fluid salt concentration.

A further observation which may be important is seen in the calculations of $R_{\text{out}}^{\text{Na}}$ in tables 7 and 8. There it will be seen that the average values for this rate are 1.63 for isotonic NaCl and 0.92 for isotonic Na_2SO_4 . The ranges of variation are rather large, but in no one of the eight cases in which adjacent

⁷ The lack of dependence of $R_{\text{into}}^{\text{Cl}}$ upon $[\text{Cl}^-]_i$ is superficially at variance with what was found for movement of sodium between ileal loops and plasma in chronic fistula dogs (1). In the earlier studies the comparison of differing sodium concentrations were made in isotonic solutions in which a divalent cation replaced varying proportions of the sodium. Under such circumstances the rate of entrance of sodium from the blood increased with the gut sodium concentration. The present studies do not duplicate that condition and therefore are not comparable.

loops were used simultaneously was there an instance in which the direction of difference was reversed. Statistical treatment of the values for the differences in individual experiments shows that a difference in the direction indicated is extremely probable. Such a difference is in spite of a higher sodium concentration gradient in the Na_2SO_4 case which would by itself reverse the difference. Since the only other difference is a substitution of a divalent for a univalent anion it seems not unlikely that that circumstance is responsible for the decrease in $R^{\text{Na}}_{\text{out}}$. The most plausible inference would seem to be that the lower permeability of the gut to sulfate than to chloride is responsible for impeding the movement of the cation.

VI. APPARENT CONCENTRATIONS OF IONS IN WATER MOVED. If the amounts of water and of particular solutes moving in a given direction, per unit of time, are known, it is a simple calculation to arrive at the "apparent" concentrations of solutes in the water moved. It seems wise to refrain from suggesting that the concentrations are real, or actual, because nothing is known with certainty about the locus or mechanism of fluid or solute transport. Therefore the calculated values will be considered as virtual or apparent until more is known about the details of transport.

The relationships in question can be visualized most readily in graphic form. In figure 1 and figure 3 are shown the calculated rates of movement of water and chloride ion from solutions of various concentrations derived from tables 3, 4 and 5. In figure 4 are presented the corresponding calculated apparent concentrations of chloride ion drawn from the same tables. It is important to note again that these comparative concentration values do not depend upon any assumptions as to identity of absorbing area in the different experiments, and especially that the differences in concentration in the outgoing and ingoing streams cannot be vitiated by any such defects in experimental situation.

It is obvious on inspection of figure 4 that the apparent concentration of chloride in the water moving from blood to gut, $[\text{Cl}^-]_{\text{into}}^{\text{a}}$ is virtually constant over the concentration range from a third to three times isotonic NaCl . On the other hand the apparent concentration in the water leaving the gut, $[\text{Cl}^-]_{\text{out}}^{\text{a}}$, rises in direct and nearly linear relation to $[\text{Cl}^-]_{\text{p}}$, over the range studied. Further, at all values of $[\text{Cl}^-]_{\text{p}}$, $[\text{Cl}^-]_{\text{out}}^{\text{a}} > [\text{Cl}^-]_{\text{into}}^{\text{a}}$, and at the higher concentration range the difference becomes very large.

It will also be noted that the mean value for $[\text{Cl}^-]_{\text{into}}^{\text{a}}$, about 27 mE per liter, is approximately a fourth of the blood plasma chloride level. This low average value is of interest, but the fact that it is derived from individual figures showing a spread from zero⁸, to 83 mE per liter is also important. Since eight analytical figures enter into each value for $[\text{Cl}^-]_{\text{out}}^{\text{a}}$ and $[\text{Cl}^-]_{\text{into}}^{\text{a}}$, it would be unrealistic to expect great reliability for any given figure. The random error in chloride analysis in the concentration range in question is 2 per cent, in radioactivity measurement under the conditions employed, the individual error may be 5 per cent, in D_2O analysis 5 per cent at the concentrations used, and

⁸ In two cases out of forty, values slightly below zero were calculated, whose significance was discussed in a footnote to table 4.

in volume measurement 5 per cent. Since, furthermore, the percentage error can increase when differences in measurements are employed it is more significant to consider a maximum absolute error. It is believed to be about 20 mE per liter for the values under consideration. However such an overall error is unlikely to occur often, and the majority of the deviations from the means observed are apt to result from real differences⁹. If the observed differences are largely real it would be concluded that different ileal preparations would show varying values for $[Cl^-]_{\text{into}}$, some of which values would approach zero. Considerable variation in ileal loop behavior from one animal to another, and in the same animal from day to day has been noted before (1, 12).

Reference to table 6, column 9, will show that in the case of isotonic $NaCl-Na_2SO_4$ solutions in ileal loops the mean value for $[Cl^-]_{\text{into}} = 20.7 \text{ } mE/l$. If the value 49.3, which is obviously out of line, is eliminated, the mean drops to 16.0 mE/l . This is a very low concentration, about one-third of that calculated for the simultaneous $[Cl^-]_{\text{out}}$.

These relationships, if correct, have obvious and important implications.

VII. FLUID CIRCUITS IN INTESTINE-BLOOD TRANSPORT. If it is permissible to employ D_2O as a tracer for water movement it follows from the data which have been presented that there is continuous movement of water both out of and into the gut. It has been pointed out that the rate R_{out}^W is far too small in relation to R_{net}^W , for hypotonic solutions, to permit the conclusion that a major share of the movement is by diffusion, or in other words that the net transport is due to normal osmosis. It has been noted, too, that the change in R_{out}^W , and in its ratio to R_{into}^W , with change in salt concentration in the gut is many times (a hundred or more) greater than would result from the difference in osmotic activity.

The evidence therefore indicates that forces other than diffusion account for the majority of the movement of water between intestinal lumen and blood. No movement can occur without a driving force and it is therefore apparent that if the foregoing conclusions are correct, the movement of water across the intestinal epithelium is brought about by some sort of forced flow. Since water movement occurs in both directions simultaneously there is obviously a functional circuit of fluid.

Simultaneous measurements of ion movements by isotopic tracers have provided the data on the basis of which it became possible to calculate the apparent concentrations of such ions in the water moved, as was noted in section VI. These values acquire importance particularly in connection with the problem of absorption from gut to blood against concentration gradients. It was noted that the mean value of $[Cl^-]_{\text{into}}$ in the presence of $NaCl$ solutions was found to be 27 mE/l , even when $[Cl^-]_o = 480 \text{ } mE/l$, and that in the presence of isotonic $NaCl-Na_2SO_4$ solutions the most likely value for $[Cl^-]_{\text{into}}$ is 16 mE/l .

Net absorption of chloride ion occurs if $R_{\text{out}}^{Cl} > R_{\text{into}}^{Cl}$. This situation must exist when $[Cl^-]_{\text{out}} > [Cl^-]_{\text{into}}$ and $R_{\text{out}}^W > R_{\text{into}}^W$. Further, it can occur

⁹ The maximum unquestionable error, evident as an irrational value, found in these studies was 6.6 mE . per liter.

even if R_{out}^W is less than R_{into}^W , providing $[\text{Cl}^-]_{\text{into}}^a$ is sufficiently much smaller than $[\text{Cl}^-]_{\text{out}}^a$. It happens that $[\text{Cl}^-]_{\text{into}}^a$ is very considerably less than $[\text{Cl}^-]_{\text{out}}^a$ under all circumstances studied in which net chloride absorption was observed. Thus it appears that this condition is essential to the occurrence of net absorption of ions. The same type of observations have been made in the case of sodium ion and the same deductions must therefore be drawn. Ions can be absorbed against large concentration gradients if $[\text{Ion}]_{\text{out}}^a \gg [\text{Ion}]_{\text{into}}^a$ and if R_{out}^W has the proper relation to R_{into}^W . The necessary relation is that the latter value be small relative to the former for most rapid absorption to occur.

The reader may be, as the authors have been, disturbed by the fact that the validity of much rather involved calculation is implicit in these conclusions. Therefore a simple check of the final deduction seems desirable, and fortunately is available. The ultimate factual deduction to be made from the studies herein reported is that, in the fluid circuit between gut and blood, the solution of salt ions entering the gut is more dilute than the one leaving it. In such a situation, unless other unmeasured solute enters, the gut solution should become progressively more dilute as time of absorption proceeds. If it began as an isotonic solution it should become hypotonic during absorption. A test of this prediction is found in earlier observations, (13, 14), and it is found to be confirmed.

When isotonic solutions are absorbed it has been found that in 20 minutes they become hypotonic to the extent of 10–40 mM NaCl, osmolar equivalents¹⁰. The exact magnitude of the change to be expected is difficult to calculate but the general extent and direction of the change constitute qualitative and perhaps rough quantitative confirmation of the above prediction. Reference to tables 4, 6, 7 and 8 will show that in the case of isotonic solutions the difference between $[\text{Cl}^-]_{\text{out}}^a$ and $[\text{Cl}^-]_{\text{into}}^a$, and the corresponding sodium values, range on the average between 20 and 40 mE/l. This difference does not necessarily correspond with the expected change in osmotic activity, because the absolute values of $[\text{Cl}^-]_{\text{out}}^a$ and R_{out}^W must also be known to make a calculation. Furthermore, the concentrations of any other osmotically active solutes in the moving fluid must be known. Since they are not, no attempt will be made to arrive at an exact quantitative prediction at this time. It is obvious, however, that the measurement of a complete balance, to be checked by osmotic activity measurements, is desirable in this connection.

The evidence on which the deduction that a forced flow of fluid occurs in a circuit between gut and blood is based, may now be summarized.

1. There is simultaneous movement of water in both directions between gut and blood.
2. The relations between the rates of total directional movement and net movement of water are incompatible with the view that simple diffusion causes the transport.

¹⁰ The papers referred to above contain a small part of the data at hand on this point. A future publication by Dr. R. R. Roepke and one of the present authors will present additional evidence bearing on this problem.

3. There is simultaneous movement of ions in both directions between blood and gut.

4. Neither the net movements nor the total directional movements of inorganic ions nor the ratios between such rates are in harmony with predictions from concentration relations alone.

5. The apparent concentration of inorganic ion in the water moved from gut to blood is approximately a linear function of ion concentration in the gut lumen over the range studied.

6. The calculations of apparent concentration of ions in the water moving out of and into the blood lead to the prediction that isotonic solutions of NaCl should become hypotonic during absorption. This prediction is in agreement with earlier observations on this point.

An alternative to the fluid flow theory would require a set of remarkable coincidences to occur to account for the facts outlined. If water and solute move independently it would be necessary to have some interdependent sets of controls about which nothing is known, because the movements are not in accord with any diffusion hypothesis which has been suggested.

The facts presented in this paper are at variance in one point from the theory of uni-univalent salt impoverishment by a fluid circuit between intestine and blood presented earlier (15). In that presentation it was suggested that the fluid leaving the gut carried uni-univalent salt in the concentration existing in the gut fluid at that time. The values for $[\text{Cl}^-]_{\text{out}}$ and $[\text{Na}^+]_{\text{out}}$ presented in this paper show that this interpretation is not possible in its simple form, because in no instance did the apparent concentrations just referred to equal the actual concentrations in the gut fluid at the same time. In general it appears to be a rule that $[\text{Cl}^-]_{\text{out}}$ bears a close and nearly linear relation to $[\text{Cl}^-]_g$ but is not identical with it.

SUMMARY AND CONCLUSIONS

Isotopic tracers have been employed in the simultaneous study of water, chloride and sodium movement between ileal lumen and blood. The rates of movement of water and the salt ions in the two directions, gut to blood, and blood to gut, and their resultant, the rate of net transport, have been derived from experimental data.

It has been observed that the rate at which D_2O leaves the gut is strongly influenced by the salt concentration. The rate with a one-third isotonic solution is more than twice that found with hypertonic solutions. The calculated rate of water movement into the gut from the blood is nearly independent of the osmotic pressure in the gut. The net water movement from gut to blood depends in general in direction and magnitude upon the osmotic activity gradient, except in the case of solutions near isotonicity, which are absorbed although the osmotic activity gradient may be zero or negative.

The rate of movement of D_2O through a cellophane membrane is not measurably influenced by the magnitude or direction of the NaCl concentration gradient across it under the conditions of observation employed.

The rate of chloride movement from gut to blood increases with increase in chloride concentration, but not in linear proportion, as might be expected in a diffusion system. The reverse movement is independent of gut chloride concentration, within the limits of error or measurement.

The rate of sodium movement from gut to blood is greater from an isotonic chloride than an isotonic sulfate solution, although the gut sodium concentration is higher in the latter case. The presence of an anion to which the gut wall is relatively impermeable apparently impedes the movement of cation.

It has been shown that the experimentally derived ratios between total directional rates and net transport rates differ as much as 200 fold from the ratios predicted on the assumption that movement is by diffusion. In certain instances, moreover, the direction of movement is the reverse of that predicted. These observations refute the commonly held belief that net water movement between ileum and blood occurs primarily because of normal osmosis. They are also incompatible with the view that concentration gradients are the major cause of chloride and sodium ion movement between the normal gut and the blood.

Calculations of the apparent concentrations of chloride ions in NaCl solutions in the water moving out of the gut show that this value is proportional to the ion concentration in the gut fluid, but that the apparent concentration in the water moving into the gut is practically independent of the concentration in the latter.

The apparent concentration of chloride and sodium ions in the outgoing fluid is greater than in the ingoing fluid, under the conditions tested. It was predicted from the latter facts that isotonic solutions undergoing absorption should become significantly hypotonic. This prediction is found to be verified by studies already published.

These observations are in harmony with the hypothesis that there is a forced flow of fluid across the intestinal epithelium in both directions simultaneously and that differences in the solute content of the water in the two streams and the relative rates of the streams determine the direction and magnitude of the net transport.

REFERENCES

- (1) VISSCHER, M. B., R. H. VARCO, C. W. CARR, R. B. DEAN AND D. ERICKSON. *This Journal* 141: 488, 1944.
- (2) INGRAHAM, R. C. AND M. B. VISSCHER. *This Journal* 114: 681, 1936.
- (3) BARBER, H. H. AND I. M. KOLTHOFF. *J. Am. Chem. Soc.* 51: 3233, 1929.
- (4) VAN SLYKE, D. D. *J. Biol. Chem.* 58: 523, 1923.
- (5) KESTON, A. S., D. RITTENBERG AND R. SCHOENHEIMER. *J. Biol. Chem.* 122: 227, 1942.
- (6) FETCHER, E. S. *Ind. and Eng. Chem.* 16: 412, 1944.
- (7) JUN-CH'UAN WANG. Thesis, University of Minnesota, 1943.
- (8) HERZFELD, K. F. AND H. M. SMALLWOOD. *Treatise on physical chemistry*. Vol. I, Chap. 3. D. Van Nostrand Co. Inc., 1932.
- (9) BERKELEY, EARL OF AND E. G. J. HARTLEY. *Proc. Roy. Soc. A.* 82: 871, 1909.

- (10) SOLLNER, K. Personal communication.
- (11) INGRAHAM, R. C. AND M. B. VISSCHER. This Journal 114: 676, 1936.
- (12) DENNIS, C. AND M. B. VISSCHER. This Journal 129: 176, 1940.
- (13) ROEPKE, R. R. AND M. B. VISSCHER. Proc. Soc. Exper. Biol. and Med. 41:500, 1939.
- (14) VISSCHER, M. B. Chemistry and medicine. p. 16, University of Minnesota Press, 1940.
- (15) INGRAHAM, R. C., H. C. PETERS AND M. B. VISSCHER. J. Phys. Chem. 42: 141, 1938.

THE SURVIVAL OF DOGS TREATED WITH NEOSYNEPHRIN DURING THE PRODUCTION OF HEMORRHAGIC SHOCK¹

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It has been generally concluded by experimental workers that pressor drugs have little or no beneficial effects on the treatment of shock. The evidence for such a conclusion is based on *a*, experiments which indicate that tremendous quantities of epinephrine can produce a shocklike state, and *b*, negative results under a wide variety of so-called shock conditions. The first type of evidence results from obviously radical and unphysiologic procedures. The efficacy of pressor drugs, in the second case, has been tested in forms of shock with variable and unpredictable percentages of survival, or under conditions where the very existence of a state of shock was questionable.

For the past two years this laboratory has employed a standardized method of producing hemorrhagic shock in dogs in which shock occurs within 6 hours in 75 to 80 per cent of the animals. Recently, the mortality rate has increased to 100 per cent by maintaining the dogs at a slightly elevated temperature (1). A large number of control observations has made it possible to predict with some accuracy the survival time of a dog in hemorrhagic shock. Thus, under such conditions, it seemed desirable to test the effect of a pressor drug on the survival time when the drug was administered during the hypotensive period. Such information should indicate in a qualitative way whether an augmented vasoconstriction acts in a beneficial or a deleterious manner on the survival of dogs in shock.

Neosynephrin, because of its prolonged action and reputed lack of effect on the myocardium (2, 3, 4), seemed to be the pressor drug of choice.

METHODS. Mongrel dogs weighing 9 to 15 kgm. were used in this study. The animals received 3 mgm. of morphine per kgm. about one-half hour previous to an anesthetic dose of sodium barbital (ca. 200 mgm./kgm.) administered intravenously. The surgical preparation consisted of inserting a tracheal cannula and cannulating the femoral artery and vein on the left side and the femoral artery on the right. Mean blood pressures were recorded from the right artery by means of a mercury manometer.

Following the procedure adopted in this laboratory for producing hemorrhagic shock (5) the dogs were bled until the blood pressure fell to 50 mm. and this pressure was maintained for approximately 90 minutes. Heparin (Liquaemin²) was used as an anticoagulant. At the end of the 50 mm. hypotensive period the blood pressure was lowered to 30 mm. by withdrawing more blood, or, if it was inclined to do so, the blood pressure was allowed to fall to 30 mm.

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spontaneously. The infusion of a 1 to 5000 solution of neosynephrin² in normal saline began within 3 minutes after the blood pressure reached 30 mm.

The rate of infusion was adjusted so that the blood pressure generally held between 50 and 75 mm. of mercury. The average rate of infusion approximated 2 cc. per minute. The elevated blood pressure was maintained for a period of 34 to 45 minutes and then allowed to fall. Immediately following the passing off of the neosynephrin effect, usually within 5 minutes, the dogs were reinfused with their own blood and the subsequent course of events observed. Those animals which gave indications of surviving for some time had their wounds closed and were placed in cages for observation. Survival times are calculated from the end of reinfusion to death. Rectal temperatures of 38 to 39°C. were maintained throughout the experiments.

RESULTS. Twelve dogs were used in this study. Eight of these survived the infusion of neosynephrin during the severely hypotensive period and ultimately died in a state of shock. One dog survived indefinitely and three died during the administration of neosynephrin. Only those dogs which survived the neosynephrin treatment and reinfusion of their own blood and subsequently died are considered as shocked animals. The results of this study are summarized in table 1.

The average survival time of the dogs whose demise came as a result of shock was 13.4 hours with a range from 1.7 to 29.7 hours. The heterogeneous nature of the group and the few numbers make further statistical treatment meaningless. However, these survival times can be compared to those obtained in a larger control group. The average survival time in a comparable control group of 23 dogs was 5.3 hours with a range from 2 to 13 hours (1).

The death of three of the dogs during neosynephrin treatment was attributed to cardiac failure. One of these exhibited ventricular fibrillation shortly after the beginning of infusion (expt. 1). Since this was the first experiment, the outcome might have been due to the too rapid administration of neosynephrin, although at the time the blood pressure was not excessively high (70 mm.). The other two deaths were characterized by cardiac irregularities developing at some time after the beginning of infusion. One of these, experiment 10, greatly resembled vagal inhibition with subsequent vagal escape. The heart completely stopped for a few seconds and then resumed beating for 10 to 15 strokes and stopped again. This occurred six consecutive times. At such low pressure the outcome was inevitable. This observation is interesting in view of the opinion expressed by Keys and Violante (4) that the bradycardia observed after neosynephrin is vagal in origin.

All of the dogs in a state of hypotension exhibited some adaptation to the neosynephrin. The rate of infusion had to be constantly increased in order to maintain a fairly constant level of blood pressure. Even increasing the infusion rate did not maintain the pressure in two of the dogs which later developed cardiac irregularities (expts. 10, 12), and in the two which had the shortest survival

² The author is indebted to Roche-Organon, Inc., for the Liquaemin, and to Frederick Stearns and Company for the Neosynephrin used in this study.

times (expts. 5, 11). The heart slowed only in a few cases after beginning the neosynephrin infusion. This is at variance with the observations of Keys and Violante (4), but in harmony with the clinical findings of Johnson (3, 6) who found that slowing of the heart did not occur when neosynephrin was administered during a hypotensive period.

Autopsies were performed on all dogs. Petechial hemorrhage in the duodenal mucosa was seen in all cases, but the severity varied considerably. The dogs which survived the longest exhibited the most marked congestion and hemor-

TABLE 1

The survival time of dogs treated with neosynephrin during the severe hypotension following hemorrhage and subsequently reinfused with their own blood

EXPT. NO.	WEIGHT	HEMOR- RHAGE, PER CFNT BODY WEIGHT	RANGE OF PRESSURE DURING NEOSYN- EPHRIN INFUSION	SURVIVAL TIME AFTER REINFUSION OF BLOOD	REMARKS
	kgm.	per cent	mm.	hours	
1	9.5	5.5	50-65	0	Ventricular fibrillation 10' after beginning neosynephrin infusion
2	9.5	7.5	50-60	29.7	Gut severely hemorrhagic
3	10.0	5.3	65-70	11.6	Gut severely hemorrhagic
4	9.5	4.7	60-75	Survived	Dog normal after 3 days. No shock
5	11.0	5.1	45-70	1.7	Couldn't maintain pressure with neosynephrin. Reinfusion started after 34' of infusion
6	11.5	4.7	70-75	8.3	Duodenum severely hemorrhagic
7	9.5	5.2	62-70	28.5	Duodenum and heart congested. Bloody exudate in peritoneal and pericardial cavities
8	9.0	5.3	50-68	3.8	Gut slightly hemorrhagic
9	10.0	5.1	60-73	20.8	Gut hemorrhagic
10	13.0	6.3	60-80	0	Dog died 30' after beginning neosynephrin. Vagal inhibition?
11	14.5	6.2	45-60	3.8	Gut slightly hemorrhagic
12	15.0	7.2	20-60	0	Cardiac failure. Gut slightly hemorrhagic. Subendocardial hemorrhage. Pericardial effusion

rhage of the upper part of the gut. Those dogs which expired only a few hours after reinfusion of their own blood and those which died of cardiac failure exhibited only a slightly hemorrhagic gut. Subendocardial hemorrhage, particularly in the left ventricle, was commonly found, but appeared more extensively in two of the dogs which died of cardiac failure (expts. 10, 12). No other significant pathology was noted.

DISCUSSION. In order to produce hemorrhagic shock in the dog, the animal must be subjected to a period of extremely prolonged hypotension (5). Apparently, the damage which eventuates in irreversible circulatory failure occurs during this period. It was hoped that an experiment of the type reported here

would give conclusive evidence as to whether increased peripheral vasoconstriction during the severely hypotensive period increases or decreases the survival time of dogs in shock.

A control series of 23 dogs subjected to hemorrhagic shock had a mortality rate of 100 per cent and an average survival time of 5.3 hours with a range from 2 to 13 hours. In the present series the average survival time of eight dogs in shock was 13.4 hours with a range from 1.7 to 29.7 hours. Three of the dogs in this small series lived longer than the maximum survival time in the much larger control series. Five exceeded the average of the control series. On the basis of this comparison, it is reasonable to conclude that an augmented vasoconstriction during the severely hypotensive period increases the survival time.

While vasoconstriction theoretically results in a decreased blood flow through peripheral tissues it is possible and probable that the rise of blood pressure elicited by neosynephrin more than compensates for the deleterious effect of further peripheral vasoconstriction by increasing the blood flow through the more vital centers. The increase in viability of the respiratory and other brain centers may allow greater insult to the peripheral tissues before death supervenes with a resulting longer survival time. At any event, the somewhat increased survival time indicates that the augmented vasoconstriction does have a slightly beneficial effect. A similar prolongation in survival time has been also reported by Kabat and Freedman (8) in traumatized cats by the use of slowly absorbed epinephrine. The question of whether such treatment is of real therapeutic value is open to debate, since no permanent improvement or recovery is apparent. Furthermore, the question of possible occurrence of myocardial damage may contraindicate its use.

Pharmacological studies on neosynephrin suggest that the drug has little effect on the myocardium of *normal* animals (3, 4, 6). In this respect it is superior to epinephrine. However, an animal in extreme hypotension can hardly be said to be normal and there is some evidence that myocardial depression occurs (8). The fact that three out of 12 dogs in this study died of cardiac failure during neosynephrin infusion is sufficient warning that caution must be exercised when administering pressor drugs during extreme hypotension. While the effect of such drugs may not be directly on the myocardium, the added strain put on the already anoxic and laboring heart by increasing the peripheral resistance may be enough to precipitate cardiac failure. This may explain the decreased tolerance to neosynephrin observed in hypotensive dogs.

SUMMARY AND CONCLUSIONS

Twelve dogs were subjected to hemorrhagic shock by controlled bleeding. Neosynephrin was infused during the severely hypotensive period (30 mm.) at a rate which maintained the level of blood pressure between 50 and 75 mm. At the end of the infusion period (34–45 min.) the dogs were reinfused with their own blood and the subsequent course of events observed. Eight of the dogs died in shock and one recovered from this treatment, while three dogs died of cardiac failure during the infusion of neosynephrin. The average survival time

of the dogs dying in shock was 13.4 hours as compared to 5.3 hours in a control series.

It is concluded that any increase in peripheral damage due to an augmented vasoconstriction is compensated for by an increased flow of blood through the vital centers resulting in an increased survival time, but no permanent improvement. However, the use of pressor drugs during severe hypotension seems to be contra-indicated because of the direct or indirect damage to the myocardium which may result from their use. The beneficial result of use of pressor drugs in the treatment of shock is questionable from a therapeutic standpoint.

REFERENCES

- (1) ANTOS, R. J. *Proc. Soc. Exper. Biol. and Med.* **56**: 60, 1944.
- (2) TAINTER, M. L. AND A. B. STOCKTON. *Am. J. Med. Sc.* **185**: 832, 1933.
- (3) JOHNSON, C. A. *Surg., Gynec. and Obstet.* **63**: 35, 1936.
- (4) KEYS, A. AND A. VIOLANTE. *J. Clin. Investigation* **21**: 1, 1942.
- (5) WERLE, J. M., R. S. COSBY AND C. J. WIGGERS. *This Journal* **136**: 401, 1942.
- (6) JOHNSON, C. A. *Surg., Gynec. and Obstet.* **65**: 458, 1937.
- (7) KABAT, H. AND A. M. FREEDMAN. *Proc. Soc. Exper. Biol. and Med.* **46**: 385, 1941.
- (8) WÉGRÍA, R., A. GUEVARA ROJAS AND C. J. WIGGERS. *This Journal* **138**: 212, 1943.

A STUDY ON THE CONVERSION OF FIBRINOGEN TO FIBRIN¹

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Many interesting observations have been reported on the relationships between fibrinogen and fibrin. In the chemical studies, no sharp differences have been observed between these two compounds; physically, one can differentiate between these proteins very easily. The nature of the chemical or physical changes in the fibrinogen molecule caused by thrombase, which converts it to fibrin, has not been established. Mellanby (1) claimed that fibrinogen is split into fibrin and a soluble globulin. These observations have been shown to be wrong (2, 3). Pressnell (4) showed that nitrogen is split off when fibrin is formed. He called thrombase a proteolytic fibrinogenase. This is undoubtedly wrong because recently Seegers (5) showed that purified thrombase contains no fibrinolytic activity. Jaques (6) found that all the fibrinogen nitrogen appears as fibrin nitrogen but that some of the fibrin remains in solution. Wöhlisch (7, 8) postulates a denaturation of fibrinogen and that the conversion is from a sol to an irreversible gel. He calls thrombase a denaturase.

Many other substances possess thrombase activity. Eagle and Harris (9), Wöhlisch and Juhling (10), Dyckerhoff and Gigante (11) showed that the proteolytic enzyme, papain, coagulated fibrinogen. Eagle (12) also demonstrated that certain proteolytic snake venoms when allowed to act on fibrinogen produced a typical fibrin clot. Chargraff and Ziff (13) found that the addition of ninhydrin to fibrinogen solutions or plasma produced typical fibrin clots. This reaction did not require either calcium or thromboplastin and appeared to parallel the action of thrombase. Following up this work Chargraff and Bendich (14) extended the series of organic compounds which clotted fibrinogen. They did not call the end product fibrin but simply coagulated fibrinogen. The following substances in low concentrations were found to coagulate fibrinogen (in order of their activity): chloramine-T, potassium 1,4-naphthoquinone-2-sulfonate, sodium 1,2-naphthoquinone-4-sulfonate, ninhydrin, alloxan and salicylaldehyde. Ferguson and Ralph (15) investigated the mechanism of fibrin formation by crystalline papain, ninhydrin and thrombase using dark field microscopy. The typical fibrin needle-like meshwork appears to be identical when either thrombase or papain is used. Ninhydrin gives a pseudo clot, a flocculent, granular precipitate forms but no fibrin needles can be seen. They state that ninhydrin is not a thrombase-like substance. Ferguson (16) in studying the action of thrombase on a purified fibrinogen solution states that the formation of fibrin under the specific influence of thrombase is not related

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to denaturative and digestive phenomena. Recently Baumberger (17) suggested the interesting hypothesis that the long crystals of fibrin that make up the clot are the result of the lining up of molecules through the formation of bridging S-S groups produced by the oxidation of the SH groups of fibrinogen by thrombase.

Hammersten (18) in 1879 suggested the possibility that in the conversion of fibrinogen to fibrin, an intermediary compound may be formed. Hiruma (19) also suggested an intermediate in the coagulation of fibrinogen in which fibrin was still in colloidal solution, but distinctly different from fibrinogen. Apitz (20) showed that when fibrinogen was either heated or treated with thrombase, a new substance was formed, which he called profibrin. It was characterized by 1, easier salt precipitation, could be flocculated out by either increasing or decreasing the sodium chloride concentration of the solution, 2, flocculation of adsorbents, i.e., barium sulfate, and 3, irreversibility of its precipitation. The purpose of this investigation is to study the factors which play a rôle in the conversion of fibrinogen to fibrin.

EXPERIMENTAL. Solubility studies. In the course of our investigations on beef fibrin, it was observed that there were some differences in the properties of fibrin obtained from defibrinated blood or recalcified plasma and that obtained when oxalated or citrated plasma is clotted with thrombase. Both fibrins were obtained as insoluble clots but their solubilities in dilute acids and alkalies were different. Hekma (21) found that fibrin gels dissolve in very dilute acids and alkalies and that alkaline solutions of fibrin may be made to gel again by addition of dilute acids. He suggests that gelation is a reversible process and believes that alkaline solutions of fibrin are identical with fibrinogen solutions. Barkan (22) found that fibrin dissolved in 0.1 N sodium hydroxide and showed the reactions of a metaprotein. Barkan and Gaspar (23) found that fibrin prepared from pure blood is insoluble in 0.02 per cent sodium hydroxide and that obtained from either oxalated or fluoride plasma is soluble in 0.02 per cent sodium hydroxide.

The fibrin obtained by defibrination of whole beef blood or by addition of calcium to oxalated or citrated beef plasma is called calcium fibrin (Ca-fibrin) and is not soluble in 0.03 per cent hydrochloric acid but swells into a transparent jelly. The fibrin obtained by clotting of oxalated or citrated beef plasma with beef thrombase is called thrombase fibrin (T-fibrin) and is soluble in 0.03 per cent hydrochloric acid. This dilute acid was used as the test solvent in all experiments; similar results can be obtained with other dilute acids. These relationships are summarized in table 1. The results indicate that beef Ca-fibrin, T-fibrin and fibrinogen are entirely different proteins with respect to their solubility in 0.03 per cent hydrochloric acid and 0.5 per cent sodium carbonate. There is a possibility that either two different mechanisms play a rôle in the conversion of fibrinogen to fibrin or that T-fibrin is an intermediate between fibrinogen and Ca-fibrin. The solubility relationships between T-fibrin and Ca-fibrin also exist in the fibrins from other species, i.e., dog and rabbit. These relationships must be a general property of the second phase of blood

clotting. T-fibrin when dissolved in 0.03 per cent hydrochloric acid can be reprecipitated by addition of 0.5 per cent sodium carbonate to pH 7.0. This precipitate comes down in a gel-like structure, similar to a clot. The precipitate which forms during the storage of oxalated or citrated beef plasma has similar properties to T-fibrin. It is soluble in 0.03 per cent hydrochloric acid and 0.5 per cent sodium carbonate but insoluble in 2 per cent sodium chloride. This protein may be T-fibrin.

TABLE 1
Solubility relationships between fibrinogen and fibrin

	2% NaCl	0.03% HCl	0.5% Na ₂ CO ₃
Beef Ca-fibrin*.....	Insoluble	Insoluble, gel	Insoluble
Beef T-fibrin†.....	Insoluble	Soluble	Soluble‡
Beef Fibrinogen.....	Soluble	Insoluble	Soluble

* Washed with distilled water.

† Washed with neutral 0.1 per cent sodium chloride and neutral distilled water, pH 7.0.

‡ Swells into a transparent gel before going into solution.

TABLE 2
Solubility of fibrins obtained by clotting oxalated beef plasma with various clotting agents

OXALATED BEEF PLASMA	REAGENT ADDED	CLOTTING TIME	SOLUBILITY IN 0.03% HCl
cc.	cc.		
5	0.2 Trypsin*	15 min.	soluble
5	1.0 Papain†	4 min.	soluble
5	0.5 Fer de Lance venom*	11 sec.	soluble
5	0.5 Clotting globulin‡	60 sec.	soluble
5	1.0 Ninhydrin*	2 hrs.	soluble
5	1.0 Rabbit serum	18 hrs.	soluble
5	0.5 Beef thrombase A§	10 sec.	soluble

* One per cent in 0.9 per cent sodium chloride.

† Cyanide activated (35).

‡ Lederle laboratories.

§ Prepared by method of Robbins (34).

Other agents can produce clots which are similar to T-fibrin. The results of experiments using trypsin, papain, Fer le lance venom, clotting globulin and ninhydrin to clot oxalated beef plasma are summarized in table 2; the same results can be obtained with citrated plasma. All the fibrins were soluble in 0.03 per cent hydrochloric acid. Ninhydrin does not give a true clot, a hard, gummy precipitate being formed. This experiment indicates that the clotting of oxalated or citrated beef plasma by thrombase or other clotting agents produces the same fibrin with respect to solubility in 0.03 per cent hydrochloric acid.

Rôle of calcium. The possibility existed that the clotting mechanism proceeded in two directions, either to produce Ca-fibrin or T-fibrin but since Ca-

fibrin is the end-product of the *in vivo* mechanism perhaps the T-fibrin is only an intermediate in the conversion of fibrinogen to Ca-fibrin.

An attempt was made to prepare T-fibrin in the presence of calcium ions. An optimum amount of calcium chloride was added to oxalated beef plasma forming a clot in 5 minutes, the fibrin was insoluble in 0.03 per cent hydrochloric acid. Thrombase A was added to a similar mixture immediately after the addition of the calcium chloride; a clot formed in 15 seconds which was also insoluble in 0.03 per cent hydrochloric acid. The addition of thrombase A to dog plasma, without an anticoagulant, or heparinized, in which calcium ions are present, produces Ca-fibrin. It was impossible to prepared T-fibrin in the presence of calcium ions which appear to be the deciding factor in the formation of Ca-fibrin.

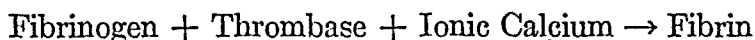
It was thought possible to change T-fibrin to Ca-fibrin by chemical means. Several grams of T-fibrin were dissolved in 25 cc. 0.03 per cent hydrochloric acid and 1 cc. 1 per cent calcium chloride added. The solution was brought to pH 7.0 with 0.5 per cent sodium carbonate and the precipitate which formed was centrifuged off. This precipitate was soluble in dilute acids and alkalies; apparently the calcium did not enter into any combination which would change T-fibrin to Ca-fibrin. The change may be a physical alteration and not a chemical one.

T-fibrin was next placed in solutions of varying concentrations of calcium chloride, 1 per cent, 0.1 per cent and 0.01 per cent. After 24 hours the fibrins were removed, washed with neutral distilled water and were found to be insoluble in 0.03 per cent hydrochloric acid. Calcium ions transform T-fibrin into Ca-fibrin. In repeating these experiments it was found that the time factor was very important. The transformation in the 1 per cent calcium chloride solution took place in approximately 10 minutes whereas the transformation in the 0.1 per cent or 0.01 per cent calcium chloride solutions required longer periods of time. Suspension of T-fibrin in beef serum will also cause the conversion to Ca-fibrin. This reaction must be due to calcium ions since the conversion will not take place when oxalated serum is used.

Calcium ions are not specific in this effect, strontium ions will also produce this conversion but barium or magnesium ions will not. This shows that strontium can replace calcium in this phase of the blood clotting mechanism. It has already been shown that strontium will activate prothrombase to thrombase (24-27). The conversion must be due to a specific ion effect; soluble calcium complexes have no effect in this relationship for citrated and oxalated plasma behave in the same way.

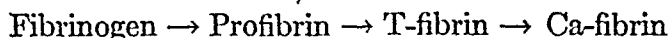
These results indicate that calcium ions are necessary for the *in vitro* formation of Ca-fibrin. It is possible that Ca-fibrin may be a calcium compound but the investigations on the calcium content of fibrin have not been conclusive. Loucks and Scott (28) found that after thorough washing of fibrin, no calcium could be detected. Mellanby and Pratt (29) showed that plasma contained 10.7 mgm. per cent and serum contained 10.6 mgm. per cent calcium. It is not difficult to conceive that 0.1 mgm. per cent calcium is enough to account

for either a calcium compound or adsorption of the calcium on the fibrin but it would be difficult to prove either point. Thorough daily washing of beef Ca-fibrin for one month followed by weekly washing for two months does not change its solubility in 0.03 per cent hydrochloric acid or 0.5 per cent sodium carbonate. It appears that once the conversion of T-fibrin to Ca-fibrin takes place, the change is irreversible. The following equation illustrates the in vitro mechanism in phase II of the blood clotting mechanism:



Mellanby and Pratt (29) observed that the coagulum formed by thrombase in the presence of calcium ions was much firmer than that formed in the absence of calcium ions. Our results indicate that this observation was correct. The calcium ions do determine the nature of the clot with respect to its physical and chemical properties. It has been shown that calcium is not necessary in the clotting of fibrinogen (30) and that thrombase acts in the presence of oxalate or citrate (31, 32). If calcium ions are necessary for the in vitro formation of Ca-fibrin, calcium ions must be necessary in phase II of the blood clotting mechanism. Another difference between T-fibrin and Ca-fibrin is shown in the studies on fibrinolysin. Tillet and Garner (33) observed that plasma fibrin (plasma plus calcium ions) is more resistant to bacterial fibrinolysin than fibrinogen fibrin (fibrinogen plus thrombase). Our results substantiate, experimentally, the presence of two different fibrin compounds in the blood clotting mechanism.

Profibrin, demonstrated by Apitz (20), is differentiated from T-fibrin and Ca-fibrin by its solubility in sodium chloride solutions; it may be an intermediate between fibrinogen and Ca-fibrin. The action of thrombase in converting fibrinogen to fibrin may be a chain mechanism involving many reactions and many intermediary compounds. The following equation may illustrate the mechanism:



Serum factor. A study on the conversion of fibrinogen to fibrin, using purified reagents, has been the aim of all investigators. The purpose of this purified system is to approximate the in vivo mechanism as closely as possible. The following experiments illustrate the relationships between fibrinogen, thrombase and calcium in phase II of the clotting mechanism.

The clotting of beef fibrinogen (34) by either beef thrombase A or B (34), or beef serum gives fibrin clots which are soluble in 0.03 per cent hydrochloric acid (T-fibrin). These fibrinogen solutions contained 0.2 per cent potassium oxalate and were adjusted to pH 7.0 with N/15 sodium hydroxide. In order to study the effect of calcium, it was first necessary to remove the oxalate by adding an amount of calcium chloride which would precipitate out all the oxalate plus 50-100 mgm. per cent calcium excess. The fibrinogen solutions were adjusted to pH 7.0 and designated as Ca-fibrinogen. The relationships between calcium, fibrinogen, thrombase A and serum are summarized in the

experiment shown in table 3. In the presence of calcium ions, the clot obtained with thrombase A was soluble in 0.03 per cent hydrochloric acid (T-fibrin). With thrombase A plus serum or serum alone, the clot was insoluble (Ca-fibrin).

TABLE 3

Solubility of fibrins obtained in a purified clotting system containing calcium ions, fibrinogen, thrombase A and serum

BEEF Ca-FI- BRINOGEN	BEEF SERUM	BEEF THROM- BASE A	SOLUBILITY* IN 0.03% HCl
cc.	cc.	cc.	
3	1.0	0	Insoluble, gel
3	0	0.3	Soluble
3	1.0	0.3	Insoluble, gel

* The mixtures were allowed to stand for 24 hours before solubility determinations were made.

TABLE 4

Solubility of fibrins obtained in a purified clotting system containing calcium ions, fibrinogen, thrombase B and serum

BEEF Ca-FI- BRINOGEN	BEEF SERUM	BEEF THROM- BASE B	SOLUBILITY* IN 0.03% HCl
cc.	cc.	cc.	
5	0.5	0	Insoluble, gel
5	0	0.1	Insoluble, gel
5	0.5	0.1	Insoluble, gel

* The mixtures were allowed to stand for 2½ hours before the solubility determinations were made.

TABLE 5

Solubility of fibrins obtained in a purified clotting system containing calcium ions, fibrinogen, thrombase A and serum protein fractions

BEEF Ca-FI- BRINOGEN	BEEF SERUM FRACTION*	BEEF THROMBASE A	SOLUBILITY† IN 0.03% HCl
cc.	cc.	cc.	
4	0 0	0.1	Soluble
4	0.5 whole serum	0.1	Insoluble, gel
4	1.0 albumin	0.1	Soluble
4	1.0 pseudoglobulin	0.1	Soluble
4	1.0 euglobulin	0.1	Soluble
4	0.5 albumin	0.1	Soluble
	0.5 pseudoglobulin		
4	0.5 pseudoglobulin	0.1	Soluble
	0.5 euglobulin		
4	0.5 albumin	0.1	Soluble
	0.5 euglobulin		
4	0.33 albumin	0.1	Soluble
	0.33 pseudoglobulin		
	0.33 euglobulin		

* Prepared by method of Hektoen and Welker (36) with modifications (2).

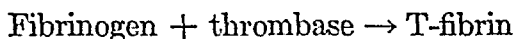
† The mixtures were allowed to stand for 2½ hours before the solubility determinations were made.

These results indicate that there is a factor in serum besides the calcium ion which is necessary for the in vitro formation of Ca-fibrin. A related observation had been made in our laboratory on the clotting of a purified fibrinogen solution with thrombase: addition of serum produces a more rapid clotting (2).

These experiments were duplicated using thrombase B, a purer fraction,

instead of thrombase A. The relationships between calcium, fibrinogen, thrombase B and serum are summarized in the experiment in table 4. The results obtained with thrombase B do not duplicate those obtained with thrombase A. The serum factor necessary to produce Ca-fibrin, when thrombase A is used, is not needed when thrombase B is used. At present, these results cannot be explained.

Serum protein fractions were used to determine what part of serum was responsible for this effect; the results of experiments using thrombase A are summarized in table 5. These results indicate that the serum factor is not any one or combination of the serum protein fractions. The fractionation of serum and isolation of the purified serum proteins in some way destroys or removes the factor responsible for the phenomenon. The following equations illustrate the in vitro mechanism in the conversion of fibrinogen to fibrin:



SUMMARY

1. Two different fibrins have been demonstrated based on their solubility in dilute acids and alkalis. Ca-fibrin is insoluble but swells into a transparent jelly in 0.03 per cent hydrochloric acid and is insoluble in 0.5 per cent sodium carbonate. T-fibrin is soluble in these reagents.

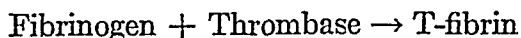
2. The precipitate which forms during the storage of oxalated or citrated beef plasma has the solubility of T-fibrin.

3. The clotting of oxalated or citrated beef plasma by thrombase, trypsin, papain, Fer de Lance venom, clotting globulin and ninhydrin produces T-fibrin; these fibrins are soluble in 0.03 per cent hydrochloric acid.

4. Calcium ions are necessary for the in vitro formation of Ca-fibrin. Calcium ions convert T-fibrin into Ca-fibrin. This effect is not a specific one, since strontium ions will also produce this conversion but barium or magnesium ions will not.

5. A serum factor besides the calcium ion is also necessary for the in vitro formation of Ca-fibrin. This factor is not present in the purified serum protein fractions.

6. The following equations illustrate the in vitro mechanism in the conversion of fibrinogen to fibrin:



REFERENCES

- (1) MELLANBY, J. *Proc. Roy. Soc., London*, s.B. **113**: 93, 1933.
- (2) WELKER, W. H. Personal communication.
- (3) WELKER, W. H., G. GILMAN AND L. HEKTOEN. *This Journal* **106**: 475, 1933.
- (4) PRESSNELL, A. K. *This Journal* **122**: 596, 1938.
- (5) SEEGER, W. H. *J. Biol. Chem.* **136**: 103, 1940.

- (6) JAKES, J. B. *Biochem. J.* 32: 1181, 1938.
- (7) WÖHLISCH, E. *Ergebn. d. Physiol.* 43: 174, 1940.
- (8) WÖHLISCH, E. *Klin. Wchnschr.* 2: 1801, 1923.
- (9) EAGLE, H. AND T. HARRIS. *J. Gen. Physiol.* 20: 543, 1937.
- (10) WÖHLISCH, E. AND L. JUHLING. *Biochem. Ztschr.* 297: 353, 1938.
- (11) DYCKERHOFF, H. AND D. GIGANTE. *Biochem. Ztschr.* 304: 334, 1940.
- (12) EAGLE, H. *J. Exper. Med.* 65: 613, 1937.
- (13) CHARGRAFF, E. AND M. ZIFF. *J. Biol. Chem.* 138: 787, 1941.
- (14) CHARGRAFF, E. AND A. BENDICH. *J. Biol. Chem.* 149: 93, 1943.
- (15) FERGUSON, J. H. AND P. H. RALPH. *This Journal* 138: 648, 1943.
- (16) FERGUSON, J. H. *J. Gen. Physiol.* 25: 607, 1942.
- (17) BAUMBERGER, J. P. *This Journal* 133: P206, 1941.
- (18) HAMMERSTEN, O. *Pflüger's Arch.* 19: 563, 1879.
- (19) HIRUMA, K. *Biochem. Ztschr.* 139: 152, 1923.
- (20) APITZ, K. *Ztschr. f. d. ges. exper. Med.* 101: 552, 1937; *ibid.* 102: 202, 1937.
- (21) HEKMA, E. *Biochem. Ztschr.* 65: 311, 1914.
- (22) BARKAN, G. *Biochem. Ztschr.* 136: 411, 1923.
- (23) BARKAN, G. AND A. GASPAR. *Biochem. Ztschr.* 139: 291, 1923.
- (24) ARTHUS, M. AND C. PAGÈS. *Arch. de Physiol. Norm. et Path.* 2: 739, 1890.
- (25) RINGER, S. AND H. SAINSBURY. *J. Physiol.* 11: 369, 1890.
- (26) MELLANBY, J. *Proc. Roy. Soc., London, s.B.* 107: 271, 1930.
- (27) KUWASHIMA, K. *J. Biochem.* 3: 91, 1923.
- (28) LOUCKS, M. M. AND F. H. SCOTT. *This Journal* 91: 27, 1929-30.
- (29) MELLANBY, J. AND C. L. G. PRATT. *Proc. Roy. Soc., London, s.B.* 128: 201, 1939-40.
- (30) HAMMERSTEN, O. *Ztschr. f. Physiol. Chem.* 22: 333, 1896.
- (31) KASTL, O. *Biochem. Ztschr.* 274: 452, 1934.
- (32) EAGLE, H. *J. Gen. Physiol.* 18: 547, 1935.
- (33) TILLET, W. S. AND R. L. GARNER. *J. Exper. Med.* 58: 485, 1933.
- (34) ROBBINS, K. C. To be published.
- (35) SCOTT, E. M. AND W. H. SANDSTROM. *Arch. Biochem.* 1: 103, 1942.
- (36) HEKTOEN, L. AND W. H. WELKER. *J. Infect. Dis.* 35: 295, 1924.

CHANGES OF CEREBRAL CIRCULATION INDUCED BY LABYRINTHINE STIMULATION

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Besides the effect upon the gastro-intestinal system, vasomotor reflexes are the most important among the vegetative reactions induced by labyrinthine stimulation. Spiegel and Démétriades (1922) demonstrated in rabbits that various types of stimulation of the labyrinth elicit a fall of blood pressure, or a multiphasic vasomotor reaction, the decline of the blood pressure sometimes being preceded, sometimes followed by a pressor effect. The depressor reaction is eliminated by paralysis of the labyrinth; it is preserved in decerebrate animals; it is a brain stem reflex mediated chiefly by the nucleus triangularis. One should expect that these reflex changes of the blood pressure are able to influence the cerebral circulation. In fact, Spiegel and Démétriades (1924) studying the cerebrospinal fluid pressure and the cerebral plethysmogram, recorded fluctuations similar to those of the systemic blood pressure. These methods, however, give only limited information regarding cerebral blood flow and are not suitable for a study of the cerebral circulation under the influence of rotation, the type of labyrinthine stimulation which is most effective in producing the physiologic stimulus, an endolymph flow in the semicircular canals. It seemed possible to approach this problem by thermo-electric methods. The use of these methods promised also to facilitate an analysis of the mechanism of the circulatory changes in the brain on labyrinthine stimulation.

METHOD. The experiments were performed on 16 cats kept under nembutal anesthesia. The board with the animal could be rotated about its longitudinal axis. In the foot end of the axis of rotation a glass tube was fixed that was connected to the recording mercury manometer. In this tube a second closely-fitting inner tube rotated with the animal and was connected to the femoral artery so that the blood pressure could be recorded during all phases of rotation. In order to study the cerebral circulation, Gibbs' (1933) thermoelectric method was used, the thermocouple usually being thrust into the parietal lobe. In the majority of the experiments, however, Schmidt's (1934, 1936) modification was employed in which the thermocouple mounted on a silver rod was cooled by keeping the rod immersed in a thermos bottle filled with ice. The thermos bottle could be moved along a groove corresponding to the longitudinal axis of the board and here fixed in any position so that a rigid connection between skull and thermocouple was secured. Control experiments on dead animals and with thermos bottle and thermocouple alone (the thermocouple being covered by a cap) showed that two types of artifacts had to be overcome. Rotation produced currents of warm air around the cooled silver rod that protruded from the thermos bottle; the result was a heating of the thermocouple imitating an increase of the cerebral blood flow. When the experiment lasted several hours, the ice melted partly in the thermos bottle so that on rotation the ice-water-mixture moved around the silver rod in the bottle; the effect was similar to that seen on slowing of the blood flow.

The first artifact was prevented by covering the silver rod between the bottle and brain

with a heat insulating rubber tube and cotton sleeve. In order to keep the ice as long as possible in close contact with the silver rod in the thermos bottle and to increase the surface upon which the ice acted, a bakelite tube was fixed coaxially around the silver rod and was divided into a series of compartments by metal discs soldered to the central silver rod. The ice was densely packed into these compartments through a slot in the bakelite tube which could then be covered to hold the ice in place. As a rule this arrangement kept the ice closely packed for 1-1½ hours; then it began to melt in the compartments that were nearest to the animal and had to be replaced. The wire connecting the thermocouple to the galvanometer circuit was held in the axis of rotation of the animal board. On rotation in one direction (as a rule up to 10 rotations) it was, of course, somewhat coiled. But control experiments showed that this produced no artifact. In successive tests the board was rotated in opposite directions, so that the wire was again uncoiled on each second test. In all graphs, upward movement of the thermocouple record means increase, downward movement slowing, of the blood flow in the parietal lobe being tested.

RESULTS. With all forms of labyrinthine stimulation used, a slowing of the cerebral blood flow was observed accompanying the fall of systemic blood pressure. For caloric stimulation a u-shaped metal cannula was introduced into the middle ear and was perfused with cold (10°C.) or hot (45-50°C.) water. The vascular effect was much better demonstrable with warm (fig. 1) than with cold water, apparently because a vasoconstrictor reaction elicited by the cold antagonized the depressor reflex.

For a study of the effect of galvanic current, monaural unipolar stimulation was employed, the testing electrode being introduced into the external auditory meatus, while an indifferent plate electrode was applied to the abdomen. In some cases the depression of the systemic pressure and accompanying retardation of the cerebral blood flow were demonstrable on anodic as well as on cathodic stimulation. In some instances this effect was obtained with the cathode only, while anodic stimulation produced even a rise of blood pressure and a parallel increase of the cerebral blood flow. This was apparently due to the fact that on application of the anode the current had to be increased up to 10 milliamperes before an effect could be noticed, and then the negative "indifferent" plate electrode produced contraction of the abdominal muscles, with subsequent rise of blood pressure.

The effect of labyrinthine stimulation upon blood pressure and brain circulation is most definite on rotation. Often it appears already during the stimulation, or the depression occurring during rotation is increased following it; or only a post-rotatory depressor reaction develops. A slight pressor reaction may precede and/or follow the fall of blood pressure and retardation of cerebral blood flow. The reaction of the cerebral vessels sometimes lags behind that of the systemic blood pressure, but in general it takes a course similar to that of the latter. The question presents itself whether the pathways carrying vasomotor impulses to the cerebral vessels mediate this reaction. The vasodilator system joining the facial and great superficial petrosal nerve (Cobb and Finesinger, 1932; Chorobski and Penfield, 1932) as well as the vasoconstrictor path using the cervical sympathetic should be considered, while fibers from the stellate ganglion to the vertebral plexus exercise no influence on pial arteries of the parietal lobe (Forbes and Cobb, 1938).

In order to interrupt the dilator tract, the middle cranial fossa was opened, the base of the temporal lobe was elevated from the petrous bone, and the ridge of this bone was cauterized in order to destroy the great superficial petrosal nerve. Or else the cerebello-pontine angle was exposed by opening the posterior atlanto-occipital membrane and removing the adjacent part of the occipital bone, and the 7th and 8th nerves were crushed in the internal auditory canal. The latter method seems more reliable than the former because the result can



Fig. 1. Blood pressure in the femoral artery, *f*, and cerebral blood flow, *c*, in the parietal lobe of cats on labyrinthine stimulation.

- Caloric stimulation (right ear, 46°C.); blood flow recorder in left parietal lobe.
- Galvanic stimulation (right ear). Blood flow recorded from left parietal lobe. Left cervical sympathetic nerve and great superficial petrosal nerve cut.
- Ten rotations.
- Five rotations; blood flow recorded from right parietal lobe; right facial and cervical sympathetic nerve cut.

s = stimulation signal, *s.o.* = simultaneous ordinates, *t* = 5 sec. intervals.

be checked not only anatomically, but also functionally, by ascertaining the loss of reflex reaction of the muscles of the face on stimulation of the cornea, conjunctiva, or other mucous membranes in the head. Interruption of these nerves, of the vasoconstrictor or the vasodilator tract alone, or of both combined, did not prevent the slowing of the circulation in the parietal cortex of the operated side that accompanied the fall of blood pressure on rotation or on galvanic stimulation of the retained 8th nerve (fig. 1).

The depressor reaction to labyrinthine stimulation is caused by a mechanism similar to the fall of blood pressure on stimulation of centripetal vagal fibers (chiefly dilatation of the vessels in the splanchnic area, Spiegel and Démétriades, 1922). One should, therefore, expect that the cerebral vessels react to labyrinthine stimulation in a similar way as to vagal stimulation. Our observations that cutting the facial nerve did not prevent the slowing of the cerebral blood flow on labyrinthine stimulation was surprising in view of the statement of Cobb and Finesinger that severance of the facial nerve prevents the reaction

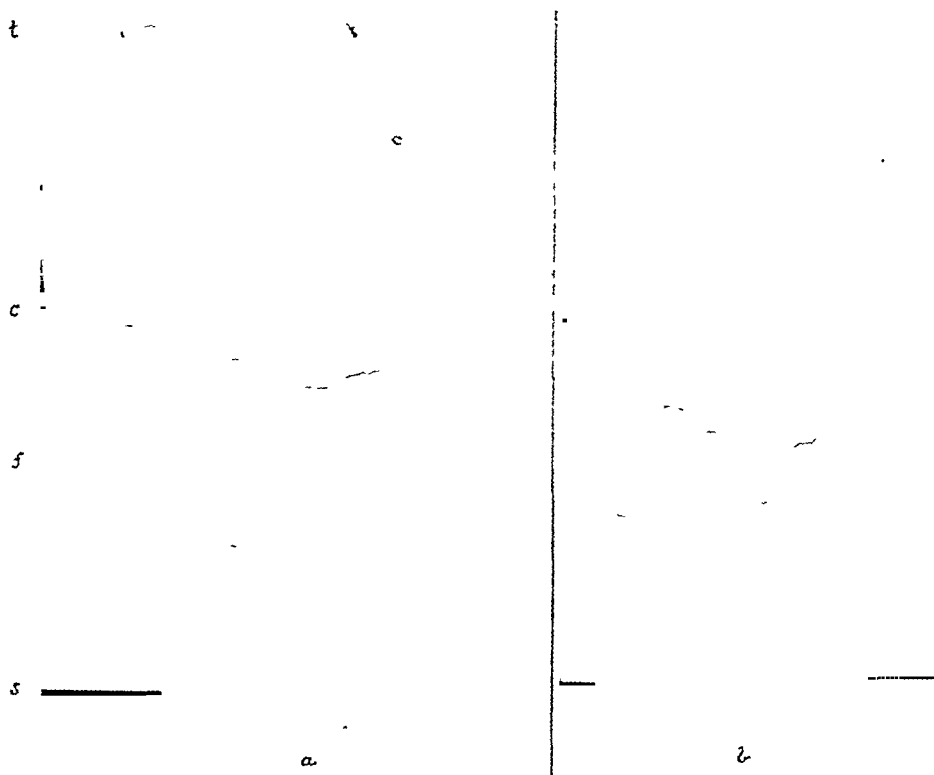


Fig. 2. Stimulation of central end of cut right vagus in cat (coil distance 6 cm.). Right facial nerve cut.

a. Blood pressure in femoral artery, *f*, and blood flow, *c*, in left parietal cortex (unoperated side).

b. Blood pressure and blood flow in right parietal cortex (side of cut facial). Other symbols as in figure 1.

of the pial arteries which normally follows stimulation of the central end of the cut vagus. Our further experiments, however, showed that also on stimulation of centripetal vagal fibers the slowing of the cerebral blood flow persists after severance of the facial or of the great superficial petrosal nerve (fig. 2).¹ A

¹ The only change in reactivity of the cerebral circulation that could be observed on labyrinthine or vagal stimulation was a poor reversibility of the retardation of the cerebral blood flow in contrast to the normal return of the blood pressure to the previous level. This delay in the recovery of the cerebral circulation was particularly pronounced when the fall in blood pressure was very marked. A technical error such as blood clot at the tip of the electrode could be excluded.

study of the literature revealed that Forbes, Nason and Wortman (1937) using the same technique of direct observation of pial arteries as Cobb and Finesinger also were unable to duplicate the results of these latter authors. They still observed dilatation of the pial arteries on stimulation of the centripetal vagal fibers after cutting the facial nerve. They suggest that slowing of the cerebral blood flow associated with fall of systemic blood pressure causes relaxation of the pial arteries by retarding the elimination of the CO_2 produced locally by the muscle fibers of the artery.

Thus our observations on labyrinthine stimulation are in agreement with those on vagal stimulation; in both cases the retardation of the cerebral blood flow is chiefly brought about indirectly through the changes in the systemic circulation; and the vasomotor tracts in the cervical sympathetic and in the facial are not necessary for the genesis of the changes in the cerebral circulation.

SUMMARY

In experiments on cats, the cerebral circulation was recorded thermoelectrically on labyrinthine stimulation. With all types of stimulation used (calorization, galvanization, rotation), a slowing of the cerebral blood flow was observed that accompanied the fall of systemic blood pressure. This reaction as well as the retardation of the cerebral blood flow on stimulation of centripetal vagal fibers are chiefly brought about indirectly through the changes in the systemic circulation, since both reactions persisted after interruption of the cervical sympathetic nerve and/or of the vasodilator tract joining the facial and great superficial petrosal nerve.

REFERENCES

- CHOROBSKI, J. AND W. PENFIELD. *Arch. Neurol. and Psychiat.* **28**: 1257, 1932.
COBB, S. AND J. E. FINESINGER. *Arch. Neurol. and Psychiat.* **28**: 1243, 1932.
FORBES, H. S. AND S. COBB. *Assoc. f. Res. in Nerv. and Ment. Dis.* **18**: 201, 1938.
FORBES, H. S., G. I. NASON AND R. C. WORTMAN. *Arch. Neurol. and Psychiat.* **37**: 334, 1937.
GIBBS, F. A. *Proc. Soc. Exper. Biol. and Med.* **31**: 141, 1933.
SCHMIDT, C. F. *This Journal* **110**: 137, 1934; **114**: 572, 1936.
SPIEGEL, E. A. AND T. D. DÉMÉTRIADIS. *Pflüger's Arch.* **196**: 185, 1922; **205**: 328, 1924.

THE EFFECT OF POSITIVE AND NEGATIVE INTRATHORACIC PRESSURE ON CARDIAC OUTPUT AND VENOUS PRESSURE IN THE DOG¹

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It has been shown (1) in the dog that the development of a large negative intrathoracic pressure is associated with a marked fall in superior vena caval pressure and a slight fall in jugular venous pressure, while the development of a large positive intrathoracic pressure is associated with a rise in peripheral venous and superior vena caval pressure. In a recent paper (2) it was shown that when the intrathoracic pressure of the barbitalized dog was increased the right auricular and peripheral venous pressure increased, and that when the intrathoracic pressure was decreased right auricular pressure decreased but the peripheral venous pressure did not change. Evidence was presented that the peripheral venous pressure remained constant when the auricular pressure decreased because the veins became partially collapsed just before entering the chest and increased the resistance to the flow of blood from the periphery to the right auricle. However, the possibility that the peripheral venous pressure remained constant, when right auricular pressure was decreased, as a result of an increased rate of flow of blood along the veins, was not ruled out.

It is generally agreed that the normal negative intrathoracic pressure aids the venous return to the heart. The breathing of air that is under a negative pressure has been shown to increase the cardiac output in the intact animal (3, 4), while the breathing of air that is under a positive pressure has been shown to decrease the cardiac output (5, 6). However, there are no quantitative data, insofar as I am aware, correlating the change in peripheral venous pressure with the cardiac output change when the intrathoracic pressure is changed. The experiments described here were performed in order to determine how much of the effect of intrathoracic pressure change on peripheral venous pressure was due to a change in cardiac output.

✓ **METHODS.** The cardiac output was determined by the direct Fick method in nine barbitalized dogs in the supine position. In each experiment the cardiac output was measured with the dog breathing oxygen from a spirometer at atmospheric pressure, at a negative pressure of 8 or 16 cm. of water, at a positive pressure of 8 or 16 cm. of water, and again at atmospheric pressure. The dog was connected to the spirometer by means of a tracheal cannula, and breathed from the spirometer at a given pressure for 10 or 15 minutes before the cardiac output determination was made. The spirometer was constructed

¹ A preliminary report of this work was given at the meeting of the American Physiological Society in Boston, 1942.

similar to the Benedict-Roth apparatus with the exception that no valves were used. The spirometer bell was carefully counterbalanced to measure the rate of oxygen consumption while the animal breathed oxygen at atmospheric pressure. Positive pressures were developed in the spirometer by adding a weight to the spirometer bell. Negative pressures were developed in the spirometer by adding a weight to the counterbalance weight. ✓

Five cubic centimeter samples of mixed venous blood were collected from the right auricle by means of a paraffin-coated cannula that passed into the right auricle by way of the right external jugular vein. The cannula remained in place throughout the experiment, which generally took from one to two hours. In order to prevent clotting a continuous injection of 0.9 per cent sodium chloride solution passed through the cannula at a rate of 1 cc./min. or less. Arterial blood was collected by cannulating a small side branch of the femoral artery. The blood was collected over mercury without contact with air by the method of Austin et al. (7) and was analyzed for oxygen by the manometric method of Van Slyke and Neill (8). Arterial blood pressure was measured in the femoral artery with a mercury manometer.

In another group of ten dogs, right auricular and peripheral venous pressures were measured simultaneously with the dog breathing from a chamber in which the pressure was varied from 20 cm. of water below to 20 cm. of water above atmospheric pressure. Right auricular pressure was measured with a water manometer by means of a cannula that passed into the right auricle by way of the right external jugular vein, and peripheral venous pressure was measured with a water manometer by means of needle puncture of the femoral, cephalic or jugular vein. All venous pressures were referred to the level of the cannula tip in the right auricle as zero. These results were described in part in an earlier paper (2).

✓ In five barbitalized dogs the cardiac output, femoral venous and right auricular pressure were measured within a few minutes of each other with the dog breathing air at atmospheric pressure, at a positive pressure of 16 cm. of water, at atmospheric pressure, at a negative pressure of 16 cm. of water, and at atmospheric pressure. The air under positive or negative pressure was breathed for 15 minutes or longer before the observations were made. After breathing air under positive or negative pressure the animal breathed air at atmospheric pressure for 30 to 45 minutes before the next control observations were made. ✓ No observations were made until 3 hours or longer after anesthesia was begun. The venous pressures were determined in a manner similar to that described above. The cardiac output was determined by a modification of Stewart's method (9). Instead of injecting hypertonic sodium chloride solution into the right heart and determining the amount of dilution of the injected salt solution in the blood of the femoral artery at an appropriate time as Stewart did, the blue dye, T 1824, was injected into the right auricle and the concentration of the dye in the blood running through the femoral artery was determined. A 0.3 per cent solution of the dye was made up in physiological saline and injected at a constant rate of 0.5892 cc. per second into the right auricle by means of a

ureteral catheter that passed into the right auricle by way of the right external jugular vein. A constant injection apparatus similar to that used by Wiggers (10) was employed. A small side branch of the femoral artery was cannulated within a few millimeters of the point where it came off of the femoral artery by means of a piece of tubing made from a 16 gauge needle. A 14 cm. length of ureteral catheter was connected to the needle tubing. Arterial blood was collected in small test tubes that were held on a revolving kymograph drum as described by Hamilton et al. (11). The drum revolved at such a rate that each tube collected blood from the artery for approximately one second. Fifteen blood samples of approximately 1 cc. each were taken for each cardiac output determination. The tubes were placed in the ice box for several hours

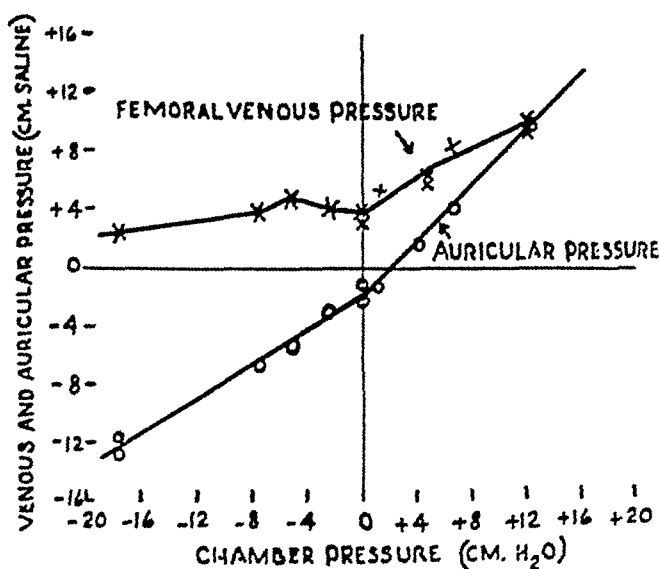


Fig. 1

Fig. 1. The effect of changing the breathing chamber pressure on right auricular and femoral venous pressure. +, above atmospheric pressure; -, below atmospheric pressure.

Fig. 2. Serum dye-concentration-time curve of arterial blood samples taken from the femoral artery. The shaded area indicates the time of injection of the dye into the right auricle.

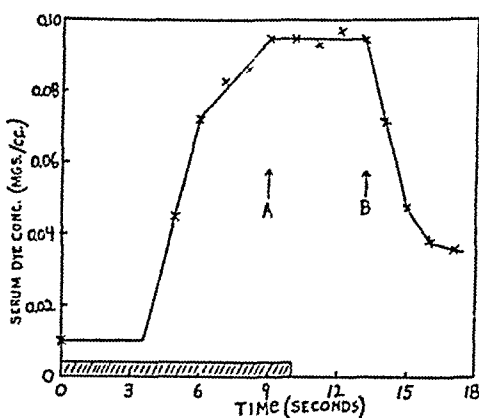


Fig. 2

and then centrifuged. The dye content of the serum was determined with a Klett-Summerson photoelectric colorimeter in a manner similar to that described by Gibson and Evans (12). Although the procedure varied somewhat in the earlier experiments the final procedure used for determining the cardiac output was as follows. A control sample of blood was drawn from the femoral artery; part of this was used to determine the hematocrit by the method of Wintrobe (13), and part to determine the dye content of the serum. A few seconds later the dye injection was started. Eight seconds after the dye injection was started the collection of arterial samples of blood was begun. The dye injection was stopped 13 seconds after it was started. Twenty-three seconds after the beginning of the dye injection the collection of blood samples was stopped. The serum dye-concentration-time curves were similar to that

shown in figure 2. The dye concentration quickly reached a level and remained at this level for 4 to 6 seconds. The serum dye concentration value when it was constant (between *A* and *B* in fig. 2) was a measure of the amount of dilution that the dye solution had undergone as a result of mixing with the blood that passed through the heart. The formula used for calculating the cardiac output in cc./min. was as follows:

$$\text{Plasma C.O.} = \frac{Q}{(C_2 - C_1)} \times 60$$

$$\text{Blood C.O.} = \frac{\text{Plasma C.O.}}{(100 - 0.915 H)} \times 100$$

Q is the number of milligrams of dye injected per second. *C*₂ is the concentration of the dye in the serum taken from the flat part of the dye-concentration-time curve. *C*₁ is the concentration of the dye in the serum sample taken just before the dye injection. These concentrations of dye are expressed in milligrams per cubic centimeter of serum. *H* is the hematocrit. Since it has been shown (14) that the hematocrit determined by the centrifuge method gives a value that is on the average 8.5 per cent higher than the true red cell hematocrit, the hematocrit has been corrected by multiplying it by 0.915.

In some cases the dye-concentration-time curves were similar to that shown in figure 2 with the exception that at about 16 seconds the dye concentration increased. It was thought that this was due to the beginning of some re-circulation of the dye.

// RESULTS. *Cardiac output.* The results of the measurements of cardiac output by the Fick method are shown in table 1. When oxygen at atmospheric pressure was breathed the cardiac output was greater at the end of the experiment than it was at the beginning in seven of eight experiments. The reason for this is not clear but it may have been due to the fact that the viscosity of the blood was less at the end of the experiment than at the beginning, as a result of the injection of a small amount of saline in washing the blood out of the auricular cannula, and the slow injection of physiological saline through the auricular cannula in order to prevent clotting. The control cardiac output in each experiment was taken as the average of the two control determinations. The deviation of the single observations from the averages ranged from ± 2 to ± 25 per cent in the eight experiments. The mean deviation from the average was ± 16 per cent.

When oxygen under a positive pressure of 16 cm. of water was breathed the cardiac output was decreased below the control output in four out of six experiments. However, in only one of these six experiments was the decrease in the cardiac output greater than the deviation of the two control output determinations from the average control. The average decrease in cardiac output was 16 per cent of the control. There was little or no change in cardiac output when oxygen under a positive pressure of 8 cm. of water was breathed. When oxygen under a negative pressure of 16 cm. of water was breathed there was an

increase in the cardiac output above the control determination in five out of seven experiments. However, in only one of the seven experiments was the increase in cardiac output greater than the deviation of the two control output determinations from the average control. The average increase in cardiac output was 13 per cent of the control. There was little or no change in cardiac output when oxygen under a negative pressure of 8 cm. of water was breathed. Thus when oxygen under a positive pressure of 8 or 16 cm. of water or a negative

TABLE 1

A							B									
Exp. No.	Vein	Pressure fall from peripheral vein to right auricle					Exp. No.	Wt. kgm.	Cardiac output (Fick method)							
		C.P. 0	C.P. +8	C.P. +16	C.P. -8	C.P. -16			C.P. 0	C.P. 0	C.P. +8	C.P. +16	C.P. -8	C.P. -16	X	
		cm. H ₂ O	% change	% change	% change	% change			l./min.	average l./min.	% change	% change	% change	% change	% deviation	
1	Femoral	5.2	-50		+104	+170	12	15.3	2.47 3.12	2.80				+4	±11	
2	Cephalic	3.8	-52		+126		13	21	3.66	3.66		-32		+37		
3	Jugular	5.4	-17	-33	+95	+150	14	18.7	3.10 3.24	3.17		+39		-2	±2	
4	Femoral	8.0	-51		+67	+180	15	11.4	1.55 2.62	2.09		-25		+9	±25	
5	Femoral	8.8	-37	-62			16	15.4	2.47 1.95	2.21		+4		+34	±12	
6	Femoral	9.0	-53		+71	+149	17	19.8	2.92 4.47	3.69		-19		+7	±21	
7	Cephalic	3.5	-71	-71	+123	+351	18	14.0	1.66 2.44	2.05		-63		0	±19	
8	Cephalic	8.6	-60	-83	+70	+146	19	11.7	1.69 2.45	2.07	+3		+3		±13	
9	Femoral	8.2	-49	-82			20	8.5	1.47 2.24	1.85	-3		-2		±21	
10	Cephalic	5.6	-68	-100												
11	Jugular	1.5	-49													
Average			-53	-72	+94	+191	Average.....				0	-16	+1	+13	±16	

A. Effect of breathing air that is under different hydrostatic pressures on the pressure fall from peripheral vein to right auricle. The increase (+) or decrease (-) of the pressure fall is given in per cent of the control. C.P., chamber pressure, is given in centimeters of water.

B. Effect of breathing oxygen that is under different hydrostatic pressures on the cardiac output. The increase (+) or decrease (-) of the cardiac output is given in per cent of the control. l., liters. In column X the deviation of the single control observations from the average is given in per cent of the average.

pressure of 8 or 16 cm. of water was breathed the cardiac output was changed on the average no more than the change in the control determinations.

Venous pressure. In each experiment twelve or more determinations of peripheral venous pressure and right auricular pressure were made. The venous and auricular pressures were then plotted on graph paper and results similar to those shown in figure 1 were obtained. From these graphs the pressure fall from the peripheral vein to the right auricle was determined when the animal breathed air at a negative pressure of 8 and 16 cm. of water, at atmospheric pressure, and at a positive pressure of 8 and 16 cm. of water. These results are given in table 1.

When air under positive pressure of 8 cm. of water was breathed the pressure fall from peripheral vein to right auricle decreased in each experiment, the average decrease was 53 per cent of the pressure fall when air under atmospheric pressure was breathed. When air under a positive pressure of 16 cm. of water was breathed similar results were obtained, the average decrease in pressure fall was 72 per cent. When air under a negative pressure of 8 cm. of water was breathed the pressure fall from peripheral vein to right auricle was increased in each experiment, the average increase was 94 per cent of the pressure fall when air under atmospheric pressure was breathed. When air under a negative pressure of 16 cm. of water was breathed similar results were obtained with the average increase in pressure fall being 191 per cent. Thus when air under a positive pressure of 8 or 16 cm. of water was breathed there was a marked decrease in the pressure fall from peripheral vein to right auricle, and when air under a negative pressure was breathed there was a great increase in the pressure fall from peripheral vein to right auricle.

Cardiac output and venous pressure. The results of these experiments in which the cardiac output was determined by a modification of Stewart's method are shown in table 2. When air under a positive pressure of 16 cm. of water was breathed the cardiac output was decreased in every case. The decrease in cardiac output was much greater than the deviation of the control outputs. The average decrease in the cardiac output was 33 per cent of the control while the average deviation of the controls was ± 6 per cent. The pressure fall from femoral vein to right auricle was decreased in every case except one, the average decrease was 64 per cent of the control.

When air under a negative pressure of 16 cm. of water was breathed the cardiac output was increased in three and decreased in two experiments. In only two cases was the increase in cardiac output greater than the deviation of the control determinations, and in these two the increase in the cardiac output was only 5 and 7 per cent. The pressure fall from femoral vein to right auricle was increased in each case when air under a negative pressure of 16 cm. of water was breathed. The average increase was 244 per cent of the control.

The breathing of oxygen under a positive or negative pressure had little effect on arterial blood pressure. When the animal was suddenly changed from breathing air at atmospheric pressure to breathing oxygen under a positive pressure of 16 cm. of water the arterial pressure fell several millimeters of mercury but rose within less than a minute to approximately the normal level. However, in experiment 18 the arterial pressure fell 35 mm. of mercury when oxygen under a positive pressure was breathed and remained at this level until positive pressure respiration was stopped.

DISCUSSION. The pressure fall from peripheral vein to right auricle is a function of the length and cross-section of the venous channels along which the blood flows from the peripheral vein to the right auricle, the volume of blood flowing along these venous channels per unit of time, the viscosity of the blood, and the hydrostatic pressure exerted by the vertical column of blood between the point where the venous pressure is measured and the right auricle. With

the length of the veins, the blood viscosity, and the hydrostatic component remaining constant, an increase in the pressure fall from peripheral vein to right auricle must be the result of an increase in the rate of flow of blood along the veins or of an increase in the resistance to the flow caused by a decrease in the cross-section of the veins.

It was shown in table 1 that when oxygen under a negative pressure of 16 cm. of water was breathed there was, on the average, only 13 per cent increase in

TABLE 2

EXP. NO.	WT. KGM.	A			B				
		Pressure fall from femoral vein to right auricle			Cardiac output (modified Stewart method)				
		C.P. 0	C.P. +16	C.P. -16	C.P. 0	C.P. 0	C.P. +16	C.P. -16	X
		cm. H ₂ O	% change	% change	l./min.	average l./min.	% change	% change	% deviation
21	22.3	3.2	-63	+290	3.44 3.69	3.57	-29	+5	±3
22	20.5	11.6	-91		3.08 3.28	3.18	-40		±3
		12.4		+90	3.28 3.34	3.30		+7	±1
23	32.0	3.6		+255	6.50 5.44	5.97		-2	±9
		5.0	0		5.44 5.11	5.28	-37		±3
24	24.6	3.6		+350	3.51 2.19	2.85		+13	±23
		5.0	-100		2.19 2.41	2.30	-28		±5
25	14.3	5.1		+236	2.82 2.72	2.77		-2	±2
		6.3	-64						
Average			-64	+244			-33	+4	±6

Same as table 1 except that the cardiac output was determined by a modification of Stewart's method.

the cardiac output, while in a similar group of experiments the pressure fall from peripheral vein to the right auricle was increased 191 per cent of the control. In table 2 it was shown that there was little, if any, increase in the cardiac output when air under negative pressure was breathed, while the venous pressure drop from femoral vein to right auricle increased greatly, the average value being +244 per cent. Thus the increase in pressure fall from peripheral vein to right auricle must have been due nearly entirely to an increase in the re-

sistance to the flow of blood along the veins when a negative pressure of 16 cm. of water was breathed; for with laminar flow in the veins it would have been necessary for the cardiac output to increase 244 per cent of the control in order to give an increase of 244 per cent in the pressure fall from peripheral vein to right auricle if there had been no change in the resistance to the flow of blood along the veins. Thus in the supine dog when air under a negative pressure is breathed the peripheral venous pressure remains constant and the right auricular pressure decreases, thereby increasing the pressure fall from the peripheral vein to the right auricle, because the resistance to the flow of blood along the veins is increased. This increase in resistance to the flow of blood is due to the fact that when air under a negative pressure is breathed the veins become partially collapsed just before entering the chest and increase the resistance to the flow of blood into the chest (2). It would appear that the collapse of the veins just before entering the chest, when large negative intrathoracic pressures are developed, is a mechanism that prevents a large amount of blood from being sucked into the chest and over-distending the heart.

Similar experiments (15) in the normal supine human indicate that the veins do not collapse just before entering the chest when air under a negative pressure of 14 cm. of water or less is breathed. This is apparently due to the fact that in normal man right auricular pressure is several centimeters of saline above atmospheric pressure (16), and as a result the intrathoracic pressure must be lowered considerably before the pressure in the veins entering the chest becomes low enough to cause them to collapse. Thus the cardiac output might be increased in man under these circumstances. However, the development of larger negative intrathoracic pressures in man, as in Muller's experiment, does appear to cause the veins to collapse just before entering the chest and this would prevent a further increase in cardiac output. It would appear unlikely that the breathing of air under negative pressure would cause much increase in the cardiac output of man when in shock or in any state where there was a decreased venous return to the heart. In these conditions the right auricular pressure tends to be lower than normal and the development of greater negative pressure in the chest would probably cause the veins to become partially collapsed just before entering the chest, increase the resistance to the flow of blood into the chest, and so prevent an increase in cardiac output.

In the dog when air under a positive pressure of 16 cm. of water was breathed the pressure fall from peripheral vein to right auricle decreased on the average 72 per cent of the control pressure fall, while the cardiac output decrease on the average was only 16 per cent of the control when the Fick method was used to determine the output. In the other group of experiments in which the cardiac output was determined by a modification of Stewart's method the average decrease in cardiac output was 33 per cent of the control, and the average decrease in pressure fall from femoral vein to right auricle was 64 per cent of the control. The decrease in pressure fall from the peripheral vein to the right auricle must have been due in part to a reduction in cardiac output, and in part to a decrease in the resistance to the flow of blood along the veins. The decrease

in resistance to the flow along the veins was probably due to the fact that the veins were dilated as a result of the high pressure in the right auricle.

It should be pointed out that the pressure fall from peripheral vein to right auricle is a function of the volume of blood flowing per unit of time along the venous channels from the point in the peripheral vein where the pressure is measured to the right auricle (in the experiment on the femoral vein these channels are the femoral vein and inferior vena cava). Only part of the cardiac output flows along these channels, and more blood flows along part of these channels than flows through the peripheral vein in which the pressure is measured. It would be possible for the rate of blood flow along one or more of these channels to change and thus change the pressure fall from peripheral vein to right auricle, even if there were no change in cardiac output. Also it would be possible for the cardiac output to change and for the rate of blood flow to change along certain venous channels and not along others. Thus the pressure fall from one peripheral vein to the right auricle might change as the result of a cardiac output change while the pressure fall from another peripheral vein might not change. However, since similar results were obtained on the femoral vein, which empties into the inferior vena cava, and the jugular and cephalic veins, which empty into the superior vena cava, it would appear that these results represent the changes that take place in all veins.

The control cardiac output determinations varied considerably in the experiments in which the Fick method was used. The reason for this is not clear but may have been due to the fact that a true sample of mixed venous blood was not obtained, for it has recently been shown (17) that blood samples taken simultaneously from different points in the right auricle may have different contents of oxygen. Also it has been pointed out (18, 19) that the cardiac output in dogs anesthetized with sodium barbital is variable and that the cardiac output during the first 90 minutes of anesthesia is generally higher than subsequent determinations. A 90 minute period following anesthesia was not allowed for the circulation to become stabilized, and only short periods of time were allowed between cardiac output determinations in these experiments. There was very little variation in the control cardiac output determinations made by a modification of Stewart's method and it is thought that these results are more accurate than those made by the direct Fick method.

SUMMARY AND CONCLUSIONS

Cardiac output was measured by the direct Fick method and by a modification of Stewart's method in dogs breathing oxygen and air at a pressure of 8 and 16 cm. of water below atmospheric, 8 and 16 cm. of water above atmospheric, and at atmospheric pressure. The control cardiac output determinations made with the Fick method showed considerable variation, while the control determinations with the modified Stewart method showed little variation. The cardiac output determinations with the modified Stewart method showed that when air under a positive pressure of 16 cm. of water was breathed the cardiac output was decreased. The average decrease was 33 per cent of the

control. When air under a negative pressure of 16 cm. was breathed there was little change in the cardiac output.

Peripheral venous and right auricular pressures were measured simultaneously in dogs breathing air from a chamber in which the pressure varied from 20 cm. of water pressure above to 20 cm. below atmospheric pressure. When air under a positive pressure of 16 cm. of water was breathed the pressure fall from peripheral vein to right auricle was decreased. The average decrease was 72 per cent of the pressure fall when air under atmospheric pressure was breathed in one group of experiments and 64 per cent in another. When air under a negative pressure of 16 cm. of water was breathed the pressure fall from peripheral vein to right auricle increased. The average increase was 191 per cent of the control pressure fall in one group of experiments and 244 per cent in another.

Since the cardiac output of the dog changes very little when air under a negative pressure of 16 cm. of water is breathed it would appear that the maintenance of a high peripheral venous pressure, when right auricular pressure is greatly decreased, is due to the fact that the veins become partially collapsed just before entering the chest and increase the resistance to the flow of blood to the right auricle. ✓

REFERENCES

- (1) ROST, E., JR. *Ztschr. f. d. ges. exper. Med.* **82**: 255, 1932.
- (2) HOLT, J. P. *This Journal* **134**: 292, 1941.
- (3) HUGGETT, A. ST. G. *J. Physiol.* **59**: 373, 1925.
- (4) MÜLLGAARD. *Fysiologisk Lungkirurgie*. Kopenhagen. Quoted by I. DE BURGH DALY. *J. Physiol.* **63**: 81, 1927.
- (5) HUMPHREYS, G. H., R. L. MOORE, H. C. MAIER AND V. APGAR. *J. Thoracic Surg.* **7**: 438, 1938.
- (6) KNOEFEL, P. K., J. P. HOLT, C. QUINN AND A. M. AMBROSE. *Federation Proc.* **3**: 76, 1944.
- (7) AUSTIN, J. H., G. E. CULLEN, A. B. HASTINGS, F. C. McLEAN, J. P. PETERS AND D. D. VAN SLYKE. *J. Biol. Chem.* **54**: 121, 1922.
- (8) VAN SLYKE, D. D. AND J. M. NEILL. *J. Biol. Chem.* **61**: 523, 1924.
- (9) STEWART, G. N. *This Journal* **57**: 27, 1921.
- (10) WIGGERS, H. C. *This Journal* **140**: 519, 1944.
- (11) HAMILTON, W. F., J. W. MOORE, J. M. KINSMAN AND R. G. SPURLING. *This Journal* **84**: 338, 1928.
- (12) GIBSON, J. G. AND W. A. EVANS, JR. *J. Clin. Investigation* **16**: 301, 1937.
- (13) WINTROBE, M. M. *J. Lab. and Clin. Med.* **15**: 287, 1929.
- (14) CHAPIN, M. A. AND J. F. ROSS. *This Journal* **137**: 447, 1942.
- (15) HOLT, J. P. *This Journal* **139**: 208, 1943.
- (16) RICHARDS, D. W., JR., A. Cournand, R. C. DARLING, W. H. GILLESPIE AND E. DEF. BALDWIN. *This Journal* **136**: 115, 1942.
- (17) HOLT, J. P. AND P. K. KNOEFEL. *Federation Proc.* **3**: 19, 1944.
- (18) JOHNSON, G. S. AND A. BLALOCK. *Arch. Surg.* **23**: 855, 1931.
- (19) BLALOCK, A. *Arch. Surg.* **15**: 762, 1927.

THE EFFECT OF *L. CASEI* FACTOR ("FOLIC ACID") ON BLOOD REGENERATION FOLLOWING HEMORRHAGE IN RATS

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Studies of the influence of nutritional factors in blood regeneration have only recently involved the use of a highly purified ration supplemented entirely with synthetic vitamins. McKibbin et al. (1) reported in 1942 that dogs fed such a diet retained an adequate capacity to regenerate blood lost in repeated hemorrhages. Anemia occurred when riboflavin was omitted from the vitamin supplement (2) but the level of thiamin did not appear to be critical (3). In the present study a severe anemia has been produced by bleeding rats fed sulfasuxidine (succinyl sulfathiazole) in a purified diet. Crystalline *L. casei* factor has been found to have a preventive and corrective action on this anemia.

Sulfasuxidine included in a purified diet fed to rats usually produces granulocytopenia rather than anemia. Hemorrhage has been used in these sulfasuxidine-fed rats as a means of demonstrating a latent erythropoietic inadequacy.

Crystalline substances which support the growth of *Lactobacillus casei* E and *Streptococcus lactis* R were isolated from liver and yeast by Stokstad (4). A new *L. casei* factor isolated from an unstated source has recently been described by Hutchings, Stokstad, Bohonos and Slobodkin (5). It has been known that certain concentrates from liver, yeast and other sources stimulated the growth of micro-organisms and exerted potent effects on the blood picture and growth of rats (6, 7, 8), chicks (9) and monkeys (10). (The term "folic acid" has often been used to denote the active principle in these concentrates.) Pfflner et al. (11) isolated a crystalline substance from liver which they termed "vitamin B_c". This prevented anemia in chicks, promoted their growth and stimulated the growth of *Lactobacillus casei* E. Daft and Sebrell (12) found that vitamin B_c and the new *L. casei* factor were both effective in correcting sulfonamide-induced blood dyscrasias in rats.

There are certain advantages in the use of rats in studies on the influence of nutritional factors in hemorrhagic anemia. Experiments can be performed with large numbers; rat nutrition has been extensively studied; and there is a simple technique available for repeated standardized bleeding of large numbers of small animals reported by Tabor, Kabat and Rosenthal (13).

METHODS. Albino rats (Wistar strain¹) were weaned at about 22 days and fed one of four highly purified diets. Of the 4 diets, one contained sulfasuxidine. This "*sulfasuxidine diet*" consisted of sulfasuxidine 1 per cent, anhydrous dextrose 70.76 per cent, casein (Labco) 18 per cent, cod liver oil 2 per cent, cottonseed (Wesson) oil 3 per cent, ferric citrate (iron 18.09 per cent) 1.16 per

¹ Osborne and Mendel rats were used in the experiment in which control diet no. 955 was fed.

cent, copper sulfate ($5\text{H}_2\text{O}$) 0.08 per cent and salt mixture no. 550 (6) 4 per cent. The "control diet no. 937" differed from the sulfasuxidine diet only in that the sulfasuxidine was replaced by an equal weight of dextrose. The "lactose diet" contained the same ingredients as the control diet no. 937 except that 15 per cent lactose replaced an equal weight of dextrose. On all 3 diets, each rat received a daily supplement of 100γ of thiamine hydrochloride, 200γ of riboflavin, 100γ of pyridoxine hydrochloride, 200γ of calcium pantothenate, 1 mgm. of niacin, 10 mgm. of choline chloride, 0.5γ of biotin (Merck) and 20γ of 2-methyl-1,4-naphthoquinone. The other diet used was the "control diet no. 955." It consisted of sucrose 68.26 grams, casein 18.0 grams, crisco 8.0 grams, ferric citrate 1.16 grams, copper sulfate ($5\text{H}_2\text{O}$) 0.08 gram and salt mixture no. 550, 4 grams. Into this diet were incorporated 1 mgm. of thiamine hydrochloride, 2 mgm. of riboflavin, 1 mgm. of pyridoxine hydrochloride, 4 mgm. of calcium pantothenate, 2 mgm. of niacin, 200 mgm. of choline chloride, 0.001 mgm. of biotin and 0.4 mgm. of 2-methyl-1,4-naphthoquinone. Twice weekly each rat received a supplement of 0.25 cc. of corn oil containing 2000 units of vitamin A and 200 units of vitamin D (Natola) and once weekly 3 mgm. of α -tocopherol in 0.03 cc. of ethyl laurate.

A special supplement of the new crystalline *L. casei* factor² of Hutchings et al. (5) was given orally by pipette to some rats for preventive or therapeutic purposes. When used for prevention of anemia, it was given as a regular daily dose of 2.5γ started 2 days before hemorrhages were begun. For correction of anemia, a daily dose of 25γ was given for each of 4 days.

In experiments in which 2, 3 or 4 conditions were under comparison, groups of 2, 3 or 4 litter mates of the same sex and closely comparable weight were used to make up the experimental groups. In the bleeding and other procedures, care was taken to study and treat litter mates alternately and similarly.

The technique used by Tabor, Kabat and Rosenthal (13) in bleeding mice was used with slight modifications for bleeding rats. The animals are restrained in cloth wrappings from which the tail protrudes. The tail is cut with a sharp knife at 1 to 2 mm. from the end and immersed in 1.3 per cent sodium oxalate solution contained in a 10 cc. graduated cylinder. The cylinder is suspended in a constant temperature water bath at 43°C . The volume of blood removed from a rat is determined by reading the increment in volume of the fluid in the cylinder. When the calculated volume of blood has been withdrawn, the tail is tied close to the end with heavy thread.

Sodium chloride (0.9 per cent) was injected intraperitoneally just prior to bleeding as a routine precaution against death during the bleeding procedure. A volume equivalent to 5 per cent of the body weight was administered. Of 68 rats bled a total of 638 times, only 2 died during the bleeding or within several hours of it. A few rats which appeared weak after the hemorrhage were given additional saline.

In all experiments but one, the volume of blood removed was equivalent to

² The crystalline material used in the present studies was furnished through the courtesy of E. L. R. Stokstad, B. L. Hutchings and N. H. Slobodkin of Lederle Laboratories.

approximately 2 per cent of the body weight determined immediately before the saline injection. In the preliminary experiment with rats fed the sulfasuxidine diet, the volume of blood removed was equivalent to approximately $1\frac{1}{2}$ per cent of the body weight. Bleedings were made every other day or three times weekly except at certain stages in the lactose experiment when more frequent bleedings were made and are so indicated.

Hemoglobin determinations on the total amount of shed blood were done by an acid hematin method, using a photoelectric colorimeter. Hematocrit values were obtained from a drop of tail blood by a micromethod using Van Allen tubes and isotonic sodium oxalate as an anticoagulant. Determinations were made routinely before each bleeding and at intervals of several days following the cessation of a series of bleedings. Hemoglobin determinations from a drop of tail blood were made with the oxyhemoglobin method of Sanford et al. (14). Total white blood cell counts were made with Trenner pipettes and counting chambers certified by the Bureau of Standards. Polymorphonuclear cells were counted directly in the chamber under a high dry lens (300 diameters).

In experiments where hemorrhagic anemia was being produced, the series of bleedings was started after 5 days' preparation on the sulfasuxidine diet in the preliminary experiment, after 10 days in the confirmatory experiment, and as specifically indicated in other experiments. The series of bleedings was terminated when hematocrit values of 20 vol. per cent or less were reached and when comparison with previous values suggested that no significant amount of regeneration was taking place. In all such experiments the bleeding period was ended simultaneously in all of the litter mates subjected to this procedure.

RESULTS. *Hemorrhage in rats fed purified diets.* The data in table 1 are from a study of 10 male rats fed control diet no. 955. Bleedings were started after a 20-day preparation on this diet. Within 5 days after the last of 10 bleedings (made during a 21-day period), the hematocrit values were found within the normal range. Weight gain was at a rate of 2.5 grams a day during this bleeding period.

In order to determine whether a change in the carbohydrate in the diet would have any effect on blood regeneration, a study was made of 4 rats which were fed a 15 per cent lactose diet. After a 10-day preparation on the diet, the rats were subjected to 22 bleedings during a 41 day period. Hematocrit values before the bleedings were started were 41, 38, 38 and 35 vol. per cent. The initial 13 bleedings³ were spaced over a period of 27 days. Two days after the thirteenth bleeding, the hematocrit values were 31, 30, 35 and 31 vol. per cent. During the following 9 days, 8 bleedings were made and 2 days later the hematocrits were 31, 28, 46 and 31 vol. per cent respectively. Three days after the 22nd bleeding, the respective hematocrit values were 40, 34, 45 and 35 vol. per cent. Weight gains during the month following the cessation of bleeding were 33, 69, 66 and 47 grams as compared with gains of 19, 20, 32 and 27 grams during the preceding month when bleedings were made.

Hemorrhage in rats fed the sulfasuxidine diet. In a preliminary experiment

³ One rat had only 10 bleedings.

observations on 7 rats indicated that a severe anemia could be produced in rats fed the sulfasuxidine diet and subjected to hemorrhage (group A). A supplement of crystalline *L. casei* factor administered to 7 litter mates (group B), had a preventive action on this anemia. In the rats given *L. casei* factor, average hematocrit values of 44 vol. per cent (range: 40-48) and 45 vol. per cent (range: 41-49) were obtained 6 and 10 days respectively after the last of 11 bleedings made during a 24 day period. In the rats not receiving this supplement, the average hematocrit values were 19 vol. per cent (range: 15-21) and 20 vol. per cent (range: 18-22) at corresponding periods of time. The average hemoglobin concentration 10 days after the last bleeding was 14.8

TABLE 1

Effect of repeated hemorrhage on 10 rats fed a purified diet

NUMBER OF HEMORRHAGE	NUMBER OF DAYS AFTER FIRST HEMORRHAGE	BODY WEIGHT (AVERAGE) (gms.)	HEMATOCRIT—VOL. %	
			Average	Range
1	0	100	47	44-51
2	3	106	39	34-46
3	5	114	41	35-43
4	7	118	38	30-41
5	9	124	37	28-44
6	11	127	42	36-45
7	14	140	42	38-46
8	16	144	39	36-44
9	19	154	44	41-48
10	21	158	41	39-46
	23	164	39	31-44
	26	166	43	36-48

Ten male Osborne and Mendel rats were fed control diet no. 955 and bled 2 per cent of their body weight 3 times weekly. Average value for total hemoglobin removed in 10 bleedings = 3.20 grams. These data were obtained in a collaborative study with Dr. Leon A. Heppel, Laboratory of Industrial Hygiene.

grams per cent (range: 14.0-15.8) in group B and 6.6 grams per cent (range: 5.3-7.6) in group A.

The findings of this preliminary experiment were confirmed in a larger experiment (table 2). In a group (A') of 10 sulfasuxidine-fed rats, the average hematocrit 6 days after the last of 6 to 9 bleedings was 14 vol. per cent (range: 9-21 vol. per cent) while in a group (B') of litter mates treated similarly but supplemented with *L. casei* factor, the average hematocrit was 35 vol. per cent (range: 29-40 vol. per cent).

Of 10 litter mates (group C') fed the same sulfasuxidine diet but not bled, anemia developed in only 1 case. The hematocrits of the other rats in group C' averaged 40 vol. per cent (range: 38-44 vol. per cent). (Severe granulocytopenia, discussed below, was noted in all the rats in group C' before death.) These hematocrit determinations were made 2 to 5 days before death (31 to

36 days after the start of the experiment). These results agree with the frequency of spontaneous anemia in a larger group of rats fed sulfasuxidine-containing purified diets in other experiments in this laboratory. Litter mates (group D') receiving the control diet no. 937 had an average hematocrit value of 40 vol. per cent at this time.

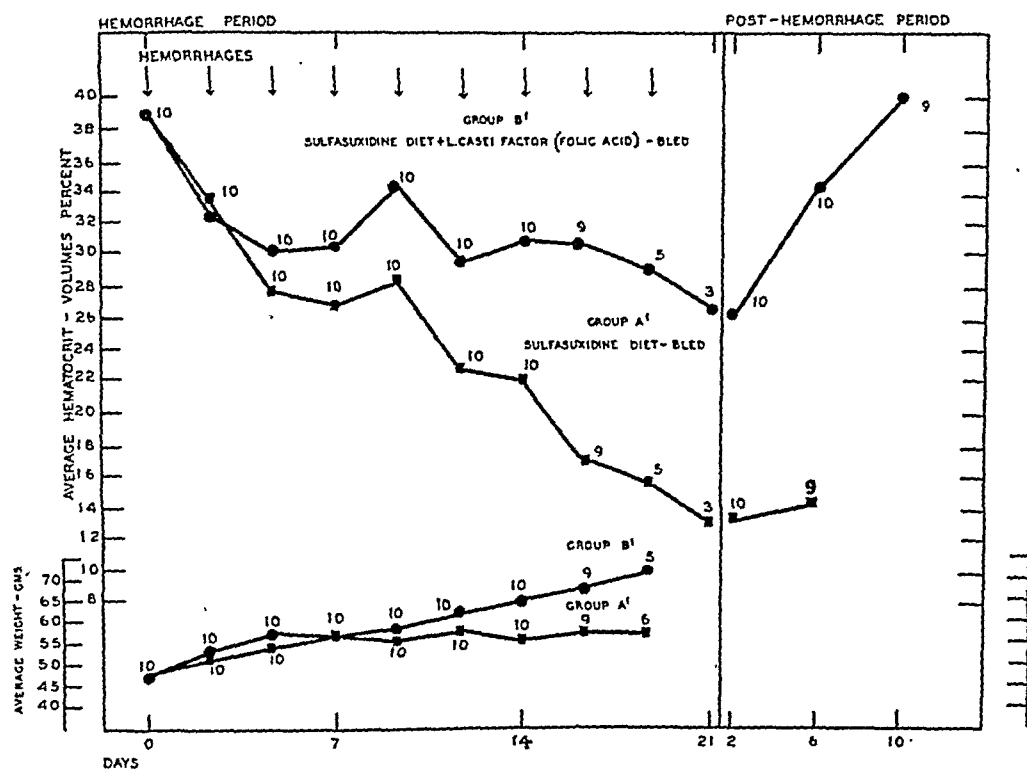


Fig. 1. Effect of crystalline *L. casei* factor ("folic acid") on the hematocrit and weight of rats fed a sulfasuxidine diet and bled three times weekly.

Group B' received $2\frac{1}{2}\gamma$ of crystalline *L. casei* factor ("folic acid") daily. Group A' (litter mates) received no supplement. Hematocrit and weight values were obtained immediately before bleeding. Each pair of litter mates was bled 2 per cent of the body weight 7 to 9 times. The numbers at each point in the figure indicate the number of animals represented in the average. Fewer animals are represented in the last 3 points in the hemorrhage period because fewer hemorrhages were necessary to reach severe anemic levels in some of the rats. The post-hemorrhage period includes all the rats. A value is not given for group A' at 10 days in the post-hemorrhage period because 5 of the rats had died and 3 had been treated with *L. casei* factor (table 2).

The curves in figure 1 represent the course of average hematocrit values during the series of bleedings. In comparing the group (A') fed the sulfasuxidine diet alone with the group (B') given a supplement of *L. casei* factor, a divergence may be noted after the second bleeding which steadily becomes more marked. The difference between the 2 groups is most striking after the cessation of bleedings, when the *L. casei* factor-supplemented rats rapidly regain normal levels while the unsupplemented rats show no evidence of recovery. A comparison of an individual rat fed the sulfasuxidine diet alone with its litter mate given a

supplement of *L. casei* factor shows a much more marked point of divergence than is indicated by a composite curve. In 10 litter mates (1A'–1B' through 10A'–10B' in table 2) which were so compared, this divergence was noted after 2, 1, 3, 4, 3, 5, 6, 2, 5 and 7 bleedings respectively.

TABLE 2

Production of anemia by bleeding rats fed the sulfasuxidine diet and prevention of anemia by L. casei factor ("folic acid")

GROUP AND DIET	RAT NUMBER	NUMBER OF BLEEDINGS	TOTAL HEMOGLOBIN REMOVED	DAYS ON SULFASUXIDINE DIET AT LAST BLEEDING	HEMATOCRIT—VOL. %				HEMOGLOBIN CONC.—GMS. %		LEUCOCYTE COUNT* 6 DAYS AFTER LAST BLEEDING	
					Initial (after 10 days on diet)	2 days after last bleeding	6 days after last bleeding	10 days after last bleeding	6 days after last bleeding	10 days after last bleeding	Total leucocytes per cu. mm.	Total polymorphonuclear leucocytes per cu. mm.
Group A' Sulfasuxidine diet	1A'	9	1.15	30	40	13	†		†		†	
	2A'	7	0.60	25	39	14	13	‡	4.9	‡	4,300	50
	3A'	7	0.71	25	41	14	13	‡	5.0	‡	1,600	0
	4A'	7	0.71	25	41	13	21	21	8.0	8.1	5,650	0
	5A'	7	0.63	25	43	9	9	†	3.8	†	1,750	0
	6A'	9	1.11	30	41	16	16	†	6.4	†	2,250	0
	7A'	9	0.70	28	40	9	8	†	2.6	†	3,500	50
	8A'	6	0.52	22	35	13	19	†	6.1	†	2,150	150
	9A'	9	0.93	28	42	12	12	‡	4.9	‡	4,500	50
	10A'	8	0.77	27	37	14	17	18	6.9	7.9	5,800	50
Averages.....			0.78		40	13	14		5.4		3,500	39
Group B' Sulfasuxidine diet + <i>L. casei</i> factor ("folic acid") 2.5 γ daily	1B'	9	1.40	30	38	23	32	39	9.9	13.2	7,500	2,500
	2B'	7	0.76	25	38	32	40	37	12.9	12.7	12,800	3,300
	3B'	7	0.95	25	39	24	34	43	11.3	14.1	9,550	2,850
	4B'	7	0.79	25	41	21	32	40	9.3	13.4	5,650	350
	5B'	7	0.82	25	41	19	29	28	9.5	11.2	5,850	300
	6B'	9	1.43	30	40	26	39	43	12.0	13.4	9,150	1,100
	7B'	9	0.88	28	37	30	38	40	11.8	13.9	10,600	1,050
	8B'	6	0.61	22	39	26	38	43	10.1	13.2	7,250	2,900
	9B'	9	1.07	28	37	33	38	43	12.4	14.9	7,800	400
	10B'	8	0.68	27	36	26	32		11.5		10,400	550
Averages.....			0.95		39	26	35	40	11.0	13.3	8,655	1,530

* Average total leucocyte counts initially (after 10 days on the diet) were 9,105 and 8,895 for groups A' and B' respectively, and the average polymorphonuclear cell counts were 1,617 and 1,741 respectively.

† Dead.

‡ Treated with *L. casei* factor. See table 4.

Bleedings of 2 per cent of the body weight were started after 10 days' preparation on the diet and carried out 3 times weekly. Rats with the same numbers in groups A' and B' were litter mates of the same sex. Rats in groups C' and D' (discussed in text) were likewise litter mates of the same sex which were given the sulfasuxidine and the control no. 937 diets respectively, but were not subjected to hemorrhage.

The weight curves in figure 1 reveal that the group (A') not supplemented with *L. casei* factor continued to gain for approximately 7 days after failure in blood regeneration was notable. The weight curves of individual rats in group A' show the same pattern as that of the average curve. Of 10 rats, all but one continued to gain in weight for 2 to 12 days after the hematocrit values began

to diverge from those of group B'. The rats in group B' continued to gain in weight to the end of the experiment.

Hemoglobin determinations made on the shed blood revealed an average total of 0.78 gram hemoglobin per rat removed from the group (A') in which anemia was produced as compared with an average total of 0.95 gram hemoglobin per rat removed from the group (B') in which it was prevented (table 2). This difference is due to the fixed procedure in this experiment of removing a volume of blood on the basis of body weight so that as rats develop anemia, relatively less hemoglobin is removed in the same volume of blood. Thus, during the first half of the experiment, rats in groups A' and B' had lost an average total of 0.47 and 0.48 gram hemoglobin per rat respectively. During the second half of the experiment when anemia was becoming manifest in group A', rats in this group lost an average total of 0.31 gram hemoglobin per rat as compared with an average total of 0.47 gram hemoglobin per rat in group B'. Thus the rats in group A' not receiving *L. casei* factor developed a severe anemia even though subjected to a smaller hemoglobin loss than the rats in group B' given a supplement of crystalline *L. casei* factor.

A severe granulocytopenia (150 polymorphonuclear cells per cu. mm. or less) was noted in 21 of 22 rats (in which counts were obtainable) getting the sulfasuxidine diet without a supplement of crystalline *L. casei* factor. No difference was observed either in the time of onset or severity of the granulocytopenia between the groups of rats that were bled and the group that was not. Severe granulocytopenia did not occur in any of the other groups (group B, group B' and group D').

Production of anemia with delayed bleedings in rats fed the sulfasuxidine diet. From the experiments described above it seemed probable that ingestion of the sulfasuxidine-containing diet created a deficiency state in which erythropoiesis was inadequate. This disturbance was seldom manifested in the hematocrit values. Therefore, the function of hemorrhage might have been to demonstrate regularly this erythropoietic inadequacy. The data in the following experiment appear to support this mechanism for the production of this hemorrhagic anemia. Three groups of litter mates were placed on the sulfasuxidine diet at weaning. Regular bleedings were started after 9 days in group I and after 18 days in group II. When both groups had been on the diet for 27 days, the average hematocrit value for group I after a bleeding period of 18 days was 21 vol. per cent, while the value for group II after a bleeding period of only 9 days was 18 vol. per cent (table 3). At this time group III, which served as a control, had an average hematocrit value of 38 vol. per cent. Bleedings were then started (after 27 days) in group III and continued in groups I and II. After 34 days on the diet, an anemia of equal severity was noted in all 3 groups even though preceded by a bleeding period of 25 days in group I, 16 days in group II, and only 7 days in group III. The amount of hemoglobin removed was in approximate proportion to the number of bleedings that were made (table 3).

Treatment of hemorrhagic anemia. Of 31 rats which developed a severe hemor-

rhagic anemia (groups A, A', I, II and III), 13 were treated with crystalline *L. casei* factor. The remaining rats which were not treated died in an average of 7 days (range: 1-13 days) after the last bleeding. During this time no significant increases in hematocrit were observed. Two rats in which treatment was started at 6 and 10 days after the last bleeding survived less than 2 days. Nine of the remaining 11 treated rats showed a striking response with a rise in hematocrit from an average level of 15 vol. per cent just prior to treatment (6 to 10 days after the last bleeding) to levels of 28 vol. per cent at 4 days after start of treatment and 41 vol. per cent at 10 days after start of treatment (table 4). Two rats showed no response at 4 days after treatment and died before the tenth day.

TABLE 3

Production of anemia with delayed bleedings in rats fed the sulfasuxidine diet

NUMBER OF DAYS ON DIET	GROUP I				GROUP II				GROUP III			
	Number of previous bleedings	Number of rats	Hema-tocrit 2 days after previous bleeding (average)	Hemo-globin removed in previous bleeding (average)	Number of previous bleedings	Number of rats	Hema-tocrit 2 days after previous bleeding (average)	Hemo-globin removed in previous bleeding (average)	Number of previous bleedings	Number of rats	Hema-tocrit 2 days after previous bleeding (average)	Hemo-globin removed in previous bleeding (average)
			vol. %	gms. %			vol. %	gms. %			vol. %	gms. %
0	0	6	40									
11	1	6	30	0.130								
14	2	6	32	0.096								
16	3	6	29	0.099								
18	4	6	27	0.098	0	6	42					
20	5	6	27	0.088	1	6	29	0.174				
22	6	6	23	0.084	2	6	23	0.110				
24	7	6	22	0.078	3	6	19	0.081				
27	8	6	21	0.074	4	6	18	0.074	0	4	38	
29	9	5	20	0.082	5	5	16	0.086	1	4	27	0.181
32	10	4	20	0.084	6	4	18	0.081	2	4	23	0.121
34	11	3	14	0.061	7	4	15	0.080	3	4	15	0.098

Rats in groups I, II, III are litter mates of the same sex and comparable weight fed the sulfasuxidine diet at weaning. Bleedings of 2 per cent of the body weight were carried out 3 times weekly.

A severe granulocytopenia was noted in each of 7 rats in which white blood cell counts were made along with hematocrit determinations before treatment. In rats which showed a positive hematocrit response, the polymorphonuclear cell count after 4 days increased from levels of 0 to 150 cells per cu. mm. to levels of 1000 to 4150 cells per cu. mm. Weight gains and increased activity accompanied the correction of the blood dyscrasias. Rats which failed to show a hematocrit rise in response to treatment likewise showed no increase in polymorphonuclear cell count.

DISCUSSION. The results of these studies show that a severe, persistent anemia may be produced regularly by bleeding rats which are fed a purified diet containing sulfasuxidine (table 2). Such an anemia was noted in only a small percentage of rats fed a sulfasuxidine diet without bleeding. When rats

were fed a purified diet without sulfasuxidine, no such persistent anemia was found even after long periods of bleeding (table 1).

In a study on the mechanism of production of this hemorrhagic anemia, it was found that the length of time during which the rats had ingested the sulfasuxidine-containing diet was more important than the number of bleedings

TABLE 4
Treatment of anemia with crystalline L. casei factor ("folic acid")

GROUP	HEMATOCRIT—VOL. %				
	Before treatment			After treatment	
	2 days after last bleeding	6 days after last bleeding	10 days after last bleeding	4 days	10 days
Treatment begun 6 days after last bleeding	14	13		23	40*
	14	13		27	38†
	12	12		28	39
	20	17		38	42
	13	9		30	37
Treatment begun 10 days after last bleeding	13	20	19	34	44
	16	15	18	14	†
	14	23	18	22	34
	16		14	15	¶
	18		20	32	50
	22		18	31	45
No treatment***	16	21	22§		
	20	21	22§		
	13	21	21**		
	13	10	7**		

* Hemoglobin conc. immediately before treatment was 4.9 gms. % and 12.6 gms. % ten days after treatment.

† Hemoglobin conc. immediately before treatment was 5.0 gms. % and 12.6 gms. % ten days after treatment.

‡ Died on 8th day after start of treatment.

¶ Died on 7th day after start of treatment.

§ Died on 12th day after last bleeding.

** Died on 13th day after last bleeding.

Data in this table are from rats with anemia (groups A, A', I, II, and III). Treatment dose was 25γ on each of 4 days. Determinations were made routinely immediately before and at 4 and 10 days after start of treatment.

*** The 18 rats with anemia which were not treated with L. casei factor died in an average of 7 days (range: 1-13 days) after the last bleeding. Values listed in this section are for rats surviving 10 days or more.

and extent of hemoglobin loss. Thus in three groups of rats fed a sulfasuxidine-containing diet from weaning, a severe anemia was developed in all at the same time, even though preceded by a bleeding period of 25 days in one group, 16 days in another, and only 7 days in the third (table 3). Therefore, it appears probable that the ingestion of sulfasuxidine in a purified diet creates a deficiency

state in which a latent anemia may become manifest by withdrawal of a sufficient amount of blood.

Supplements of crystalline *L. casei* factor ("folic acid") had a preventive action on the production of such an anemia in rats fed a sulfasuxidine diet and subjected to hemorrhage (table 2). Litter mates, which had not been given this supplement and developed anemia, responded to the later administration of the supplement in corrective doses (table 4). From an average value of 15 vol. per cent before treatment, the hematocrit rose to 28 vol. per cent 4 days later and then to 41 vol. per cent on the tenth day after the beginning of treatment. Hematocrit levels in anemic rats which were not treated showed no significant increases or decreases until death.

It should be emphasized that the doses of *L. casei* factor which were found to have preventive and corrective actions on this hemorrhagic anemia do not necessarily represent the optimal dosage levels. It is not unlikely that increased effectiveness might result from the use of higher dosages or from the concurrent administration of other factors. Comparison of the regenerative capacity of animals on a purified diet (table 1) with that of sulfasuxidine-fed rats supplemented with *L. casei* factor ("folic acid") (table 2) supports these possibilities.

Hematocrit values were obtained regularly during the series of bleedings to which the sulfasuxidine-fed rats were subjected. In a comparison of the hematocrit values of an unsupplemented rat with those of its litter mate given a supplement of crystalline *L. casei* factor, the point at which the two diverge may be seen clearly. As time went on, this difference became greater until finally when the series of bleedings was terminated, the hematocrit value of the unsupplemented rat had reached a low level from which it did not rise, while the supplemented rat quickly achieved normal values which it maintained. These data indicate that *L. casei* factor exerts an important influence on red blood cell regeneration after hemorrhage in sulfasuxidine-fed rats.

It is noteworthy that a definite indication of failure in blood regeneration occurred while the rats were still gaining in weight and were alert and active.

The powerful blood regenerative capacity possessed by rats was shown in the study where a purified diet was fed (table 1). Bleedings equivalent to 2 per cent of the body weight were made 10 times in a 21-day period. Within 5 days after the last bleeding, each rat reached near normal hematocrit levels. If the whole blood volume is considered to be approximately 8 per cent of the body weight (15), then these rats lost and regenerated their complete red blood cell volume about every 9 days. This study does not indicate the maximum regenerative capacity.

By bleeding rats during the development of a deficiency state, we have been able to demonstrate an erythropoietic inadequacy which would otherwise have remained latent. The use of an easy technique for repeated standardized bleeding of small animals has permitted the study of large numbers of rats. This approach and technique serving as a "load test" may find useful application in the detection of latent hemopoietic dysfunction in a variety of deficiency, toxic and disease states.

SUMMARY

Severe anemia may be produced regularly in rats fed a sulfasuxidine-containing, purified diet and subjected to hemorrhages. In rats fed the same diet but not bled, anemia occurs in only a small percentage of the cases.

Development of the anemia is more dependent on the length of time that the diet has been fed than on the length of the bleeding period.

Crystalline *L. casei* factor ("folic acid") has been found to have a preventive and corrective action on this hemorrhagic anemia.

Rats fed a purified diet alone did not develop anemia when bled to the same extent as the sulfasuxidine-fed rats.

It is probable that the ingestion of sulfasuxidine in a purified diet creates a deficiency state in which repeated hemorrhages may make a latent erythropoietic inadequacy manifest.

The use of hemorrhage as a "load-test" is suggested for the detection of latent hemopoietic inadequacy. The availability of a simple technique for repeated standardized bleeding of large numbers of small animals encourages the use of such a test.

REFERENCES

- (1) McKIBBIN, J. M., A. E. SCHAEFER, C. A. ELVEHJEM AND E. B. HART. *J. Biol. Chem.* 145: 107, 1942.
- (2) SPECTOR, H., A. R. MAASS, L. MICHAUD, C. A. ELVEHJEM AND E. B. HART. *J. Biol. Chem.* 150: 75, 1943.
- (3) MAASS, A. R., L. MICHAUD, H. SPECTOR, C. A. ELVEHJEM AND E. B. HART. *Arch. Biochem.* 4: 105, 1944.
- (4) STOKSTAD, E. L. R. *J. Biol. Chem.* 149: 573, 1943.
- (5) HUTCHINGS, B. L., E. L. R. STOKSTAD, N. BOHONOS AND N. H. SLOBODKIN. *Science* 99: 371, 1944.
- (6) SPICER, S. S., F. S. DAFT, W. H. SEBRELL AND L. L. ASHBURN. *Pub. Health Repts.* 57: 1559, 1942.
- (7) KORNBERG, A., F. S. DAFT AND W. H. SEBRELL. *Science* 98: 20, 1943.
- (8) AXELROD, A. E., P. GROSS, M. D. BOSSE AND K. F. SWINGLE. *J. Biol. Chem.* 148: 721, 1943.
- (9) MILLS, R. C., G. M. BRIGGS, JR., C. A. ELVEHJEM AND E. B. HART. *Proc. Soc. Exper. Biol. and Med.* 49: 186, 1942.
- (10) SASLAW, S., H. E. WILSON, C. A. DOAN AND J. L. SCHWAB. *Science* 97: 514, 1943.
- (11) PFIFFNER, J. J., S. B. BINKLEY, E. S. BLOOM, R. A. BROWN, O. D. BIRD, A. D. EMMETT, A. G. HOGAN AND B. L. O'DELL. *Science* 97: 404, 1943.
- (12) DAFT, F. S. AND W. H. SEBRELL. *Pub. Health Repts.* 58: 1542, 1943.
- (13) TABOR, H., H. KABAT AND S. M. ROSENTHAL. *Pub. Health Repts.* 59: 637, 1944.
- (14) SANFORD, A. H., C. SHEARD AND A. E. OSTERBERG. *Am. J. Clin. Path.* 3: 405, 1933.
- (15) BECKWITH, J. R. AND A. CHANUTIN. *Proc. Soc. Exper. Biol. and Med.* 46: 66, 1941.

THE EFFECT OF ANOXIC AND ANEMIC ANOXIA ON THE MOTILITY OF THE SMALL INTESTINE AND THE INFLUENCE OF AN EPINEPHRINE-POTENTIATING-AGENT¹

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It has been shown that even severe degrees of anoxic anoxia have no appreciable effect on the propulsive motility of the small intestine in the dog. On the other hand the motility of the stomach (1) and that of the large intestine (2) are decreased by even moderate degrees of this type of anoxia. Since the small intestine has a similar innervation to that of the stomach it would be expected that anoxic anoxia would inhibit the motility of the small intestine the same as it did the stomach.

In order to study this phase of the problem further it was thought worthwhile to premedicate the animals with an epinephrine-potentiating-agent, such as cocaine, before subjecting them to anoxic anoxia. It was felt that the combined action of anoxia and an epinephrine-potentiating-agent (cocaine) should produce a noticeable decrease in the propulsive motility of the small intestine. It had been shown previously (3) that cocaine alone had no appreciable effect on the propulsive motility of the small intestine in the normal dog.

It has been shown recently in our laboratory (4) that in dogs a hemorrhage equal to 3 per cent of the body weight caused a significant acceleration of the propulsive motility of the small intestine. This finding was unexpected, because it is generally felt that hemorrhage stimulates the sympathetic division of the autonomic nervous system and the motility, therefore, should have been decreased. In this instance, too, it was deemed of interest to administer an epinephrine-potentiating-agent such as cocaine, to an animal which previously had been hemorrhaged.

Since the effect of an epinephrine-potentiating-agent (cocaine) had been studied in combination with anoxic anoxia and with anemic anoxia on the propulsive motility of the small intestine, it seemed logical to study also the combined effect of anemic and anoxic anoxia (cocaine was not administered in this study). This was of particular interest, because it was found previously that hemorrhage caused an increase in motility of the small intestine, whereas anoxic anoxia had no effect.

METHODS. *a.* The effect of an epinephrine-potentiating-agent and anoxic anoxia on the motility of the small intestine. Matched pairs of dogs were used, one to serve as the control animal and the other as the experimental. The experimental animal was given by stomach tube 50 cc. of 10 per cent powdered

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charcoal in a 10 per cent aqueous solution of gum acacia. Cocaine (10 mgm./kgm.) was given intramuscularly immediately. Five minutes later the animal was placed in a low-pressure chamber and subjected to a pressure of 246.8 mm. Hg, which corresponds to an approximate altitude of 28,000 feet. At the end of 30 minutes, counting from the time the animal was intubated, the animal was removed from the low-pressure chamber, a fatal concentration of ether administered, the small intestine removed, slit open and the distance traversed by the charcoal mixture measured.

The control animals in lieu of cocaine were given an intramuscular injection of normal saline solution. They were not, of course, subjected to anoxic anoxia, but otherwise the same procedure was followed.

b. The effect of an epinephrine-potentiating-agent and anemic anoxia on the motility of the small intestine. Matched pairs of dogs were used also in these experiments. One animal served as a control while the other was subjected to a hemorrhage.

The femoral artery was exposed under light ether anesthesia and an amount of blood equal to 3 per cent of the body weight was withdrawn. The wound was closed and the animal allowed 6 hours to recuperate from the effects of the anesthesia and the immediate effects of the hemorrhage. It was then given 50 cc. of the powdered charcoal-acacia mixture by stomach tube and immediately cocaine (10 mgm./kgm.) was given intramuscularly. At the end of 30 minutes the animal was sacrificed and the distance the charcoal-mixture had traversed the small intestine was measured.

The control animal was treated the same as the experimental except that an intramuscular injection of normal saline solution was given in lieu of cocaine and although the femoral artery was exposed and ligated no blood was withdrawn.

c. The combined effect of anoxic and anemic anoxia. In this instance practically the same procedure was used as described under b. The animals were not, however, given cocaine following intubation of the charcoal-mixture, but subjected to anoxia (a barometric pressure of 294.4 mm. Hg) four minutes later. At the end of 30 minutes (from the time of intubation) the small intestines were removed and the distance measured which the charcoal had traversed.

RESULTS. The combined effect of an epinephrine-potentiating-agent and anoxic anoxia on the propulsive motility of the small intestine is shown in table 1. The results clearly indicate that the propulsive motility of the small intestine is significantly decreased.

Table 2 shows the combined effect of cocaine and hemorrhage (3 per cent of the body weight) on the propulsive motility of the small intestine. These data also show that the motility is markedly decreased.

Table 3 shows the combined effect of anemic and anoxic anoxia. The data show that when the animal is subjected to these two types of anoxia the propulsive motility of the small intestine is not significantly affected.

It will be noted that the total lengths of the small intestine of the control animals was approximately the same as the experimental. This was especially

true of the data in tables 1 and 2. Because of this the actual number of centimeters traversed by the charcoal-acacia mixture was used instead of percentage figures.

TABLE 1

Effect of cocaine administration combined with anoxic anoxia (246.8 mm. Hg.) on the propulsive motility of the small intestine

CONTROL			EXPERIMENTAL		
Body weight	Length of intestine	Distance traversed by charcoal	Body weight	Length of intestine	Distance traversed by charcoal
kgm.	cm.	cm.	kgm.	cm.	cm.
9.21	225	190	8.96	225	90
10.30	257	210	5.67	172	117
6.60	186	186	8.62	251	96
5.10	198	184	7.79	234	124
10.30	237	210	5.67	172	117
8.19	245	222	8.50	214	119
4.93	203	199	6.21	249	181
8.84	267	158	10.10	291	63
5.39	200	173	4.96	200	120
11.80	301	250	7.88	245	47
9.41	213	213	8.87	252	199
Ave.: 8.19	230	200*	7.57	228	116*

* Difference = 84 cm.; $t = 5.5006$; $p = <0.001$. (t and p according to Fisher)

TABLE 2

Effect of cocaine administration combined with anemic anoxia (hemorrhage 8% of body weight) on the propulsive motility of the small intestine

CONTROL			EXPERIMENTAL		
Body weight	Length of intestine	Distance traversed by charcoal	Body weight	Length of intestine	Distance traversed by charcoal
kgm.	cm.	cm.	kgm.	cm.	cm.
10.7	403	272	9.27	236	90
5.87	201	183	7.99	259	78
8.45	258	175	11.5	318	79
9.69	220	201	12.8	274	82
3.49	163	131	4.88	222	56
16.0	349	248	12.9	329	57
Ave.: 9.03	266	202*	9.89	273	74*

* Difference = 128 cm.; $t = 5.8996$; $p = <0.001$.

DISCUSSION. It has been recognized for a long time that anoxia may influence the autonomic nervous system. Cannon has held for many years that the sympathetico-adrenal system plays an important rôle in the adaptation of

an animal to anoxia. Sawyer *et al.* (5) in 1933 reported that cats which had had the greater part of their autonomic nervous system removed could not withstand anoxic anoxia nearly as well as normal cats. It is held by Monge (6) and by Aste (7) that residents of high altitudes show a hypertonus of the autonomic nervous system.

Recently Gellhorn and his associates have shown that a variety of agents such as anoxic anoxia and metrazol (8), heat and cold (9), cocaine and bulbo-capnine (10) all are capable of stimulating both the sympathetic and parasympathetic nerves. It is pointed out by these workers, however, that as a rule the action of the sympathetic nerves predominates over that of the parasympathetic.

In view of the work of Gellhorn and his colleagues our findings are of interest. We observed that although anoxic anoxia alone does not produce a

TABLE 3

The combined effect of anoxic anoxia (294.4 mm. Hg) and anemic anoxia (hemorrhage 3% of body weight) on the propulsive motility of the small intestine

CONTROL			EXPERIMENTAL		
Body weight	Length of intestine	Distance traversed by charcoal	Body weight	Length of intestine	Distance traversed by charcoal
<i>kgm.</i>	<i>cm.</i>	<i>cm.</i>	<i>kgm.</i>	<i>cm.</i>	<i>cm.</i>
11.2	367	182	7.03	303	157
4.31	181	166	5.13	199	182
9.13	336	119	8.33	262	176
6.29	238	184	10.5	358	278
14.4	249	249	13.6	251	251
11.5	289	69	12.7	272	172
16.8	223	196	18.0	333	60
7.20	230	80	8.16	223	223
8.05	231	231	8.36	203	81
6.12	219	182	8.96	230	177
Ave.: 9.20	256	166*	10.08	273	186*

* Difference = 20 cm.; $t = 0.60901$; $p = >0.20$.

decrease in propulsive motility of the small intestine of the dog, it does do so if the animal is premedicated with an epinephrine-potentiating-agent like cocaine. This result is not unexpected since it is generally felt that anoxic anoxia stimulates the sympathetic division of the autonomic nervous system. This is indeed a nice example of synergistic action in the animal organism.

If an epinephrine-potentiating-agent is administered following a hemorrhage the picture is somewhat more complicated. Hemorrhage, that is, anemic anoxia, produces an acceleration of the propulsive motility of the small intestine (4) presumably by stimulation of the parasympathetic nerves. It may probably be assumed, however, that anemic anoxia like anoxic anoxia is capable of stimulating both the sympathetic and parasympathetic nerves. When cocaine is administered following a hemorrhage its epinephrine-potentiating qualities

stimulate the sympathetic division of the autonomic nervous system and anemic anoxia now produces a decrease in the propulsive motility of the small intestine rather than an acceleration as it did when cocaine was not administered. The combined effects of anoxic anoxia and the epinephrine-potentiating-agent, too, could be considered as a synergistic action, that is, their combined action overshadows the stimulation of the parasympathetic nerves and hence hemorrhage no longer produces an acceleration of the propulsive motility of the small intestine.

As previously mentioned anoxic anoxia has no appreciable effect on the propulsive motility of the small intestine in the dog, whereas anemic anoxia produces a significant increase in motility. The data in table 3 show that when these two types of anoxia were used together there was no significant change in the motility of the intestine.

If we assume that anemic anoxia like anoxic anoxia is capable of stimulating both the sympathetic and parasympathetic nerves, it would be expected that some synergistic action might occur and that stimulation of the sympathetic nerves would predominate over that of the parasympathetic. That this was true, in part at least, is shown by the fact that a combination of anoxic and anemic anoxia prevented the acceleration of the propulsive motility of the gut ordinarily produced by anemic anoxia alone.

SUMMARY

The combined effect of an epinephrine-potentiating-agent (cocaine) and anoxic anoxia and with anemic anoxia was studied on the propulsive motility of the small intestine of the dog. It had been established previously that neither anoxic anoxia nor cocaine alone had any appreciable effect on the motility of the small intestine. It had been shown, however, that hemorrhage equal to 3 per cent of the body weight caused a significant acceleration of the motility of the small intestine.

It was observed that if the animals were premedicated with cocaine and then subjected to anoxic anoxia, there was a statistically significant decrease in the propulsive motility of the small intestine. Further, if cocaine was given to an animal which had suffered a hemorrhage (equal to 3 per cent of the body weight), anemic anoxia no longer produced an acceleration but rather a statistically significant decrease in propulsive motility.

The interpretation of these findings is that in the first instance cocaine produced a synergistic action with anoxic anoxia; the sympathetic nerves were stimulated and the motility of the small intestine was decreased. In the second instance, that is, following hemorrhage, cocaine again produced a synergistic action with the sympathetic nerves, so that during anemic anoxia the action of these latter nerves predominated over that of the parasympathetics.

When animals were subjected to combined anoxic and anemic anoxia, (without the influence of cocaine) the first named type of anoxia by stimulating the sympathetic fibers prevented the acceleration of the propulsive motility of the small intestine ordinarily produced by anemic anoxia alone.

REFERENCES

- (1) VAN LIERE, E. J., D. H. LOUGH AND C. K. SLEETH. Arch. Int. Med. 58: 130, 1936.
- (2) VAN LIERE, E. J., D. W. NORTHUP AND J. C. STICKNEY. This Journal 140: 119, 1943.
- (3) VAN LIERE, E. J., D. W. NORTHUP AND J. C. STICKNEY. This Journal 141: 462, 1944.
- (4) VAN LIERE, E. J., D. W. NORTHUP AND J. C. STICKNEY. This Journal 142: 260, 1944.
- (5) SAWYER, M. E. M., T. SCHLOSSBERG AND E. M. BRIGHT. This Journal 104: 184, 1933.
- (6) MONGE, C. M. ET AL. "Fisiologia andina—circulacion," An. de la Facultad de ciencias medicas (Lima) 17: 1, 1935.
- (7) ASTE. Cited by C. M. MONGE, *ibid*.
- (8) FELDMAN, J., R. CORTELL AND E. GELLHORN. This Journal 131: 281, 1940.
- (9) GELLHORN, E. AND J. FELDMAN. This Journal 133: 670, 1941.
- (10) FELDMAN, J., R. CORTELL AND E. GELLHORN. Proc. Soc. Exper. Biol. and Med. 46: 157, 1941.

SOME FACTORS INFLUENCING PHOSPHATE TURNOVER IN MUSCLE¹

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Previous studies in which radioactive phosphorus (P^{32}) was used as a tracer have shown that the resting metabolism of striated muscle involves a cycle of phosphate interchanges in which inorganic phosphate, phosphocreatine (PC), the labile phosphate groups of adenosine triphosphate (ATP), and fructose-6-phosphate (FP) take part (8, 9). Glucose-6-phosphate (GP) does not enter into the cycle, nor is the cycle utilized to supply the energy for contraction (5, 6). These tracer studies have also indicated that the absorption of glucose by the muscle cell takes place through the formation of GP on the membrane, with subsequent penetration into the cell interior of only the glucose portion of the molecule (8). The work to be presented here consists of a study by the same tracer technique, of the effects of prolonged activity, recovery from such activity, and the administration of glucose, on the metabolism of the phosphorus compounds of muscle.

The principal findings in these experiments are: prolonged activity does not influence either the uptake of P^{32} or its distribution between the various P compounds; recovery from such activity does increase the turnover of P significantly, especially in the post-absorptive state; the administration of glucose to the post-absorptive animal depresses the turnover of PC and ATP, but does not influence the turnover of GP. These latter effects are seen both in resting muscle and in recovery from contraction. In addition, further evidence has been obtained in favor of the mechanism of glucose absorption previously postulated.

Cats were used as the experimental animals, as in the previous studies. Ordinarily they were fed once daily, late in the afternoon. Those which were used the morning following such regular feeding were considered to be in the post-absorptive state. In another group, food was withheld for one day before the animals were used. These were considered to be fasting at the time of the experiment.

The induction of anesthesia and preparation of the gastrocnemius muscles were carried out in the usual way. When glucose was given, 30 cc. per kgm. of 5 per cent solution was injected intraperitoneally, half an hour after the subcutaneous administration of the disodium phosphate containing the P^{32} . Stimulation was by means of condenser discharges through the sciatic nerve, at the rate of 1 per second for 15 minutes, against moderate initial tension. In the recovery experiments, a two-hour period was allowed after the cessation of stimulation. In every case, one muscle was left in the resting state throughout. Four hours

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after the injection of the P^{32} , the two muscles were frozen in the usual manner. Trichloroacetic acid filtrates were prepared, and the P compounds isolated from the filtrates. The phosphate present as PC, and as the two acid-labile groups of ATP, were separated in the manner previously described (9). The hexosemonophosphate fraction was subjected to fractional hydrolysis with acid (8) to obtain fractions representing FP and GP. All phosphate precipitates were converted to $MgNH_4PO_4$, and measurements of radioactivity made on the material so obtained by the procedure used in the previous studies. In the tables, all measurements of relative radioactivity have been recalculated to the basis of one million counts per minute injected per kgm. body weight, as of the day of counting the samples.

Effects of prolonged stimulation. This procedure was carried out only on post-absorptive animals, since previous work has shown that the high P^{32} content of the GP in fasted animals represents principally material adsorbed on the cell membrane rather than intracellular material, whereas in post-absorptive animals, the P^{32} in this substance, as well as in the PC and ATP, represents phosphorus which is principally, if not entirely, intracellular. Furthermore, the nature of the experimental conditions chosen limits the number of points on which significant data can be obtained. It has previously been established (9) that four hours after the injection of P^{32} the normal resting metabolism results in equal P^{32} contents of PC and ATP. It therefore becomes impossible under the present conditions to determine whether interchange takes place between these two compounds in contraction. The points which can be studied are: first, whether the prolonged activity results in an increased total uptake of P^{32} , and second, whether the activity brings about interchange between the PC and ATP on the one hand, and the FP and GP on the other. The data of table 1 show that neither increased uptake of P^{32} nor interchange between the nitrogenous and the carbohydrate P compounds has taken place. These data confirm and extend the findings of Flock and Bollman (1), who found no increased uptake of P^{32} by the ATP of muscles of rats stimulated at the rate of 3 twitches per second for periods up to 60 minutes. Under their conditions, a large part of the ATP underwent breakdown in the early part of the contraction period, whereas with the stimulation rate of 1 per second, there is no breakdown of this compound.

Recovery from prolonged activity. In their experiments on rats, Flock and Bollman found an increased P^{32} content of the ATP after recovery. The present results, in table 1, confirm their observation, also show that the effect is not limited to this substance, but is general for all the organic P compounds present. The percentage increase in uptake of P^{32} is about the same for all the compounds except GP. Since it has been shown that FP in cat muscle is formed only from P derived from PC or ATP, whereas the formation of GP is an independent process (8), the experimental finding can best be interpreted as that of an acceleration in recovery of the type of metabolic process which characterizes the resting state. In the light of the concept generally held today, that phosphorylation is associated with oxidation, the finding tends to support the view expressed by the author several years ago (10), that the increased oxygen consumption and heat produc-

tion by muscle in the post-contraction period represent a gradual return of the stimulated tissue to the resting metabolic level, rather than the oxidative recovery from anaerobic contraction first postulated by Hill in 1913 (2), and restated as recently as 1940 (3, 4).

TABLE 1

Effect of stimulation, recovery and glucose administration on P^{32} turnover in muscles of cats in post-absorptive state

Values are expressed as counts per minute per mgm. P, calculated to the basis of one million counts per minute injected per kilogram body weight.

PHOSPHOCREATINE	ADENOSINE TRIPHOS- PHATE	FRUCTOSE-6- PHOSPHATE	GLUCOSE-6 PHOSPHATE	PHOSPHO- CREATINE	ADENOSINE TRIPHOS- PHATE	FRUCTOSE-6- PHOSPHATE	GLUCOSE-6 PHOSPHATE
Resting				Stimulated			
119	98	61	77	99	102	41	54
130	132	76	59	138	125	69	77
170	143	—	47	204	160	105	44
157	150	133	73	104	115	—	76
Av. 144	131	90	64	136	126	72	63
				Stimulation and recovery			
146	145	73	58	308	282	100	72
137	140	64	45	207	177	126	73
208	172	110	67	263	234	168	83
124	115	75	55	157	174	128	65
Av. 154	143	81	56	234	217	131	73
After glucose							
67	58	55	38	89	85	75	74
131	76	51	37	147	118	41	59
89	87	67	70	90	101	71	75
80	87	67	60	124	108	78	70
R 46	51	32	47				
L 39	56	17	30				
R 105	99	—	38				
L 75	92	50	94				
Av. 79	76	48	53	113	103	66	70

Effects of glucose administration. There is a marked difference in the effects of glucose administration on P turnover in fasting and in the post-absorptive state. In resting muscles of post-absorptive animals, glucose causes a decrease in the P^{32} level reached by all the organic P compounds except GP. Presumably this signifies a decrease in the metabolic turnover of these compounds, or, stated in different terms, a partial suppression of the phosphorylation cycle.

This effect of glucose is seen, not only in the resting state, but also in recovery

from contraction (table 1). Here also, GP alone of the four compounds is not affected. The average P^{32} content of the PC, ATP and FP of the resting muscles after glucose administration is practically one-half that of these compounds in animals not given glucose; similarly, the P^{32} contents of these compounds in the recovering muscles is one-half as great after glucose as when no glucose is given. In the case of GP, on the other hand, the average figures, both in rest and recovery, are the same with and without glucose administration. Since in the cat FP is formed only from P introduced into the cell interior as PC or ATP, these findings again serve to emphasize that the phosphorylation cycle is concerned only with resting metabolism and that GP does not enter the cycle.

In the fasting animal, glucose is without effect on the turnover of PC and ATP (table 2). This may be related to the effect which has frequently been observed, of the failure of a single administration of glucose to raise the R.Q. in a fasting animal, presumably because the insulin content of the pancreas is very low in fasting. It has recently been shown (7) that insulin does increase the P turnover in the muscles of fasting animals given glucose. This cannot be the complete explanation, however, for it leaves unaccounted for the decreased turnover that glucose causes in the post-absorptive animal, which is capable of responding by an increased insulin output to the glucose stimulus.

The administration of glucose to the post-absorptive animal has a quantitatively greater effect on the turnover of PC than of ATP. In the case of the latter, the uptake of the tracer is reduced to the same level as in the fasting animal; the turnover of PC, on the other hand, is reduced to levels well below those found in fasting. This difference in effect strengthens the view expressed elsewhere (9) that the formation of PC on the membrane is a process independent of the formation at ATP in the same site, even though the compounds are inter-related in the metabolic processes of the cell interior.

It is impossible to establish from the data at hand whether glucose administration has any effect on the recovery increase in turnover that was found in the post-absorptive state. It must be pointed out, however, that such data would have been valid only with respect to PC and ATP. The high P^{32} content of the GP in the fasting animals has been shown (8) to consist principally of material adsorbed on the cell membrane rather than that which has entered the cell by metabolic turnover. In the work referred to the conclusion was drawn that this adsorbed material represented GP formed on the membrane as the first reaction in the absorption of glucose, and that in the absorption only the glucose portion of the molecule entered the cell interior. On this basis the P^{32} content of the GP during active glucose absorption does not, unfortunately, give a measure of the rate at which glucose is being absorbed. Rather it gives a picture of the instantaneous situation on the membrane. For this reason, the failure of the GP fraction to show any consistent increase in P^{32} content during recovery from activity under glucose administration, may be an artefact. That is, it would be expected that under the conditions given, glucose absorption would be going on actively. However, the time selected for the recovery period may not have

been the optimum one; it is quite possible that, had the muscles been sampled earlier in recovery, a much higher P^{32} content would have been found. To establish this point would require a very prolonged series of experiments in which muscles were sampled at different durations of recovery.

TABLE 2

Effect of glucose administration and of stimulation and recovery on P^{32} turnover in muscles of cats in fasting state

Values are in same units as in table 1

PHOSPHOCREATINE	ADENOSINE TRIPHOS- PHATE	FRUCTOSE-6- PHOSPHATE	GLUCOSE-6 PHOSPHATE	PHOSPHO- CREATINE	ADENOSINE TRIPHOS- PHATE	FRUCTOSE-6- PHOSPHATE	GLUCOSE-6 PHOSPHATE
Resting							
R 108	82	—	—				
L 117	67	—	—				
R 64	50	—	—				
L 80	51	—	—				
R 136	91	—	—				
L 137	79	—	—				
R 173	113	—	—				
L 182	117	—	—				
R —	75	31	114				
L —	84	14	167				
R —	52	19	170				
L —	77	25	219				
R —	78	60	228				
L —	65	56	252				
R —	70	45	217				
L —	63	—	208				
Av. 125	76	36	208				
After glucose							
Resting				Stimulation and recovery			
210	106	—	665	191	133	—	830
87	63	—	402	106	95	—	342
88	84	50	610	108	96	59	855
52	72	103	385	70	76	50	396
R 67	64	—	495				
L 76	70	—	434				
R 158	82	72	590				
L 88	73	38	528				
Av. 103	76	66	514	119	100	55	606

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SUMMARY AND CONCLUSIONS

1. The effects of prolonged stimulation, recovery, and glucose administration on the uptake of P^{32} by the phosphorus compounds of muscle have been studied on cats in the fasting and post-absorptive states.

2. The uptake of P^{32} by phosphocreatine, the labile phosphate groups of adenosine triphosphate, and fructose-6-phosphate is greater in the post-absorptive state than in fasting.

3. Prolonged contraction is without effect on either the uptake of P^{32} by any of the acid-soluble organic phosphorus compounds of muscle, or its distribution among them.

4. In recovery from prolonged activity, there is a marked increase in the metabolic turnover rate of all the P compounds present, as evidenced by a higher P^{32} content.

5. In the post-absorptive state, the administration of glucose reduces the metabolic turnover rate of phosphocreatine, adenosine triphosphate, and fructose-6-phosphate, but not that of glucose-6-phosphate; these effects are seen both in resting muscle and in that recovering from prolonged activity.

6. In the fasting state, the administration of glucose does not affect the metabolic turnover rate of phosphocreatine and adenosine triphosphate.

7. The findings tend to support the view that an oxidative phosphorylation cycle is involved in the resting metabolism of muscle, but not in the metabolism of muscular contraction, and that glucose-6-phosphate does not take part in this cycle.

REFERENCES

- (1) FLOCK, E. V. AND J. L. BOLLMAN. J. Biol. Chem. 152: 371, 1944.
- (2) HILL, A. V. J. Physiol. 46: 28, 1913.
- (3) HILL, A. V. Proc. Roy. Soc. B 127: 297, 1939.
- (4) HILL, D. K. J. Physiol. 98: 207, 1940.
- (5) SACKS, J. This Journal 129: 227, 1940.
- (6) SACKS, J. This Journal 140: 316, 1943.
- (7) SACKS, J. Science 98: 388, 1943.
- (8) SACKS, J. This Journal 142: 145, 1944.
- (9) SACKS, J. AND C. H. ALTSHULER. This Journal 137: 750, 1942.
- (10) SACKS, J. AND W. C. SACKS. This Journal 112: 116, 1935.

CONCENTRATION OF POTASSIUM IN SERUM AND RESPONSE TO VAGAL STIMULATION IN THE DOG¹

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Many years ago Howell (1, 2) observed that "an increase in concentration of potassium salts within certain limits increases the sensitivity of the (terrapi) heart to vagus stimulation." No mammalian experiments were reported, although in a subsequent article the statement is made that results with perfused cats' hearts support those on the turtle (3). Hering (4) has also reported experiments on the dog which suggest that the relationship between potassium and the vagus is also to be found in the mammal. Subsequently attention was directed away from this action of potassium by the demonstration that acetylcholine is apparently the chief mediator of peripheral parasympathetic impulses (5). Within recent years, however, other evidence has developed which points to an intimate physiological association between acetylcholine and the potassium ion. For example, they are both concerned in some way with the transmission of excitation at the myoneural junction in skeletal muscle (6, 7, 8) and in the production of the action current in nerve (9, 10, 11, 12). The present experiments examine the possibility that potassium may generally enhance the effectiveness of parasympathetic stimulation in the mammal. Attention has been confined wholly to effects obtained within the restricted range of concentrations of serum potassium compatible with life and normal behavior.

METHODS. Seventeen dogs under nembutal anesthesia were employed. Changes in heart rate and in auriculo-ventricular (A-V) conduction time were recorded by the electrocardiograph following a variety of physiological procedures carried out at various levels of the serum potassium. These included 1, stimulation of the distal end of the ligated right vagus; 2, pressure on the carotid sinus and on the larynx; 3, elicitation of the carotid sinus reflex by the transient hypertension of adrenalin injection; and 4, the intravenous injection of 0.05 to 0.10 mgm. of acetyl- β -methylcholine chloride. The influence of acetyl- β -methylcholine on the motility of the stomach, recorded by means of a balloon and tambour system, was also studied. The concentration of potassium in serum was increased either acutely by the slow intravenous infusion of an isotonic solution of potassium chloride, or more gradually by the auto-intoxication with potassium which follows bilateral ureteral ligation (13). In certain experiments of the latter type these various procedures were repeated following intravenous injection of a solution of calcium chloride, in order to determine the effect of an increased concentration of calcium in serum. Potassium and calcium determinations were made by methods previously described (14, 15).

¹ This work was supported by a grant from the Associate Committee on Army Medical Research of the National Research Council of Canada.

In experiments involving direct stimulation of the vagus nerve, constancy of stimulation was achieved by use of shielded electrodes applied to the distal portion of the ligated nerve with as little dissection as possible. That constancy of stimulation was attained was demonstrated by the fact that stimulation regained its original degree of effectiveness, when potassium levels returned to normal, several hours after the control stimulation.

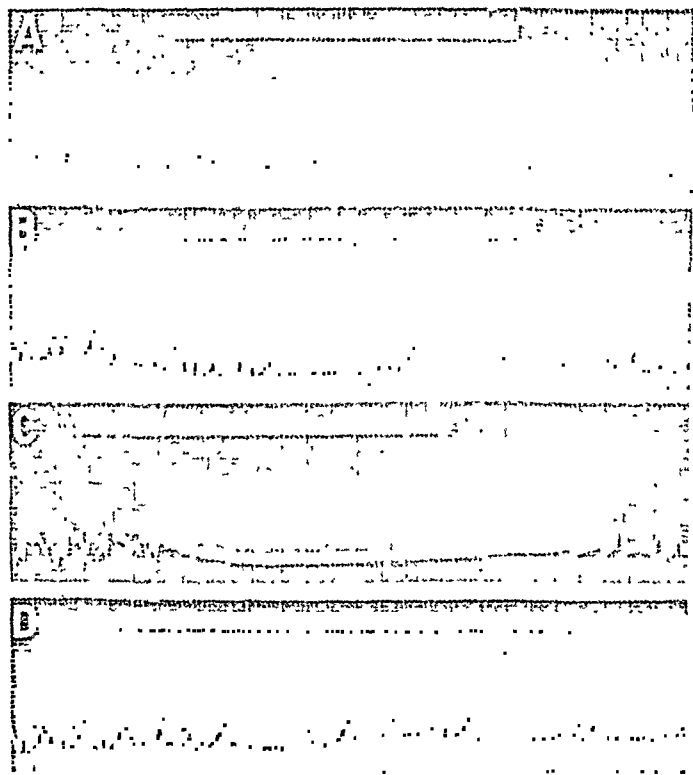


Fig. 1

Fig. 1. March 16, 1944. Dog. Nembutal anesthesia. Effect of increasing serum potassium concentrations on the bradycardia induced by electrical stimulation of distal end of cut right vagus. White bars indicate interval of stimulation. A: control electrocardiogram, serum potassium 3.3 m.eq. per liter. B and C: successive stimulation as the serum potassium rose to 8.0 m.eq. per liter. D: stimulation some time after the infusion of potassium chloride had been stopped, and the serum potassium had fallen to 4.6 m.eq. per liter. Note the increased response at higher levels of serum potassium.

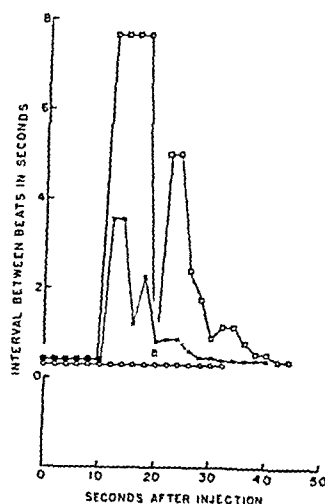


Fig. 2

Fig. 2. January 14, 1944. Dog. Nembutal anesthesia. Heart rate following intravenous injection of 0.5 cc of acetyl- β -methylcholine chloride (1:5,000) at levels of serum potassium of 6.3 (circles), 8.3 (crosses), and 9.3 m.eq. per liter (squares). The response is much greater at higher levels of serum potassium.

RESULTS. In all experiments the sensitivity of the heart to vagal stimulation was increased in the presence of an elevated serum potassium (fig. 1) without regard to the means whereby vagus discharge was invoked, whether direct, or reflexly via the carotid sinus or laryngeal reflexes. The response to intravenous injection of acetyl- β -methylcholine chloride was equally increased (fig. 2). This increased sensitivity was manifest after elevations of serum potassium as small as 1.0 m.eq. per liter, and became progressively more marked as higher levels

were reached. Results obtained when serum potassium increased spontaneously in anuria were the same as when potassium was injected. Significant changes were noted at concentrations of potassium in serum which had produced only a slight elevation in the amplitude of the T waves, but had not altered the normal rate of the heart nor disturbed intracardiac conduction. In certain experiments relatively small doses of acetyl- β -methylcholine chloride, 0.05 mgm. in a 25-kgm. dog, were without influence on the auricular rate either before or after

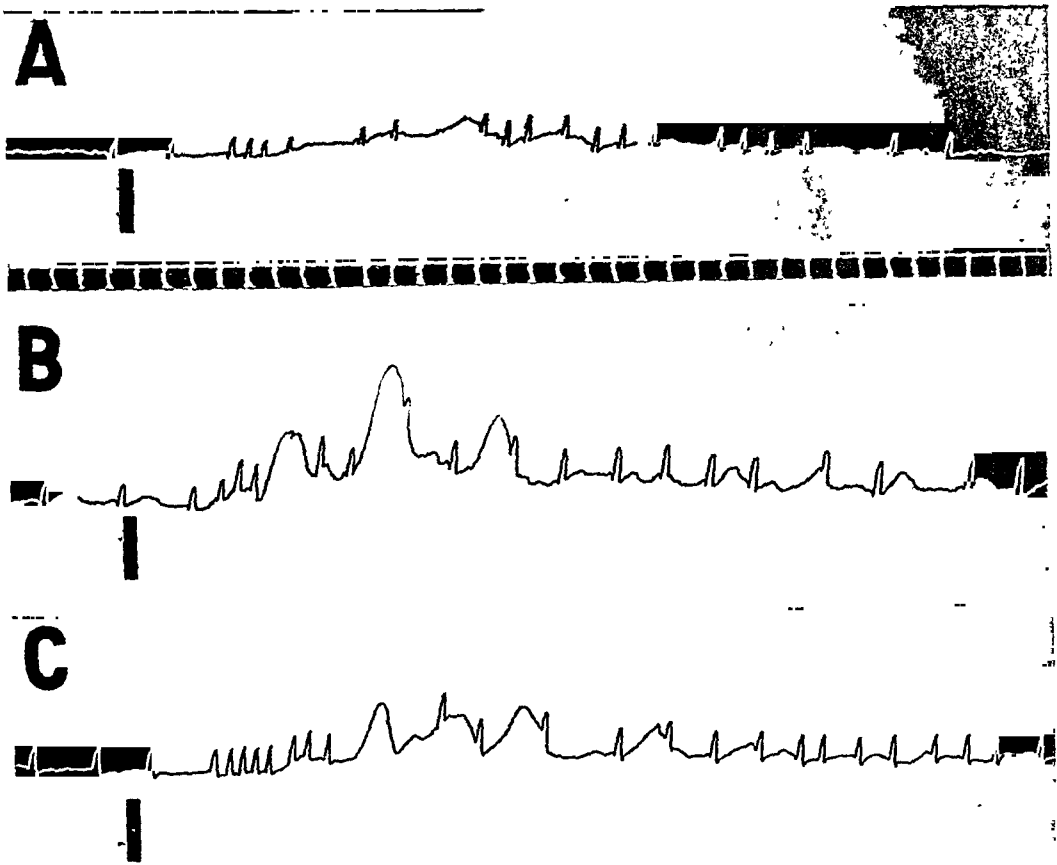


Fig. 3. March 1, 1944. Dog. Nembutal anesthesia. Influence of acetyl- β -methylcholine chloride, 0.5 cc. of 1:5,000 intravenously, on gastric motility at different levels of serum potassium, as recorded by balloon and tambour. Bars indicate moment of injection. A: control, serum potassium 4.0 m.eq. per liter. B: just after infusion of potassium chloride, serum potassium 6.5 m.eq. per liter. C: two hours after termination of infusion, serum potassium 5.0 m.eq. per liter.

elevation of the serum potassium. Auriculoventricular block and subsequent ventricular arrest were the sole cardiac effects of the drug. With increase in serum potassium the duration of the block was prolonged. The increased sensitivity of the heart to vagal stimulation with a rising concentration of serum potassium was reversible when the concentration of potassium was permitted to decline. With decreasing concentration of serum potassium the responsiveness to stimulation with acetyl- β -methylcholine chloride was consistent with the current level of serum potassium, and both returned to normal simultaneously.

A similar augmentation in the response of the stomach to intravenous injection of acetyl- β -methylcholine chloride followed elevation in the serum potassium concentration (fig. 3). The salivary response to this drug was also magnified in the presence of an elevated serum potassium. These effects were also completely reversible.

Increase in the concentration of serum calcium, although it was associated with some reduction in the level of serum potassium, was found to produce a further, very marked, increase in the cardiac response to acetyl- β -methylcholine (fig. 4).

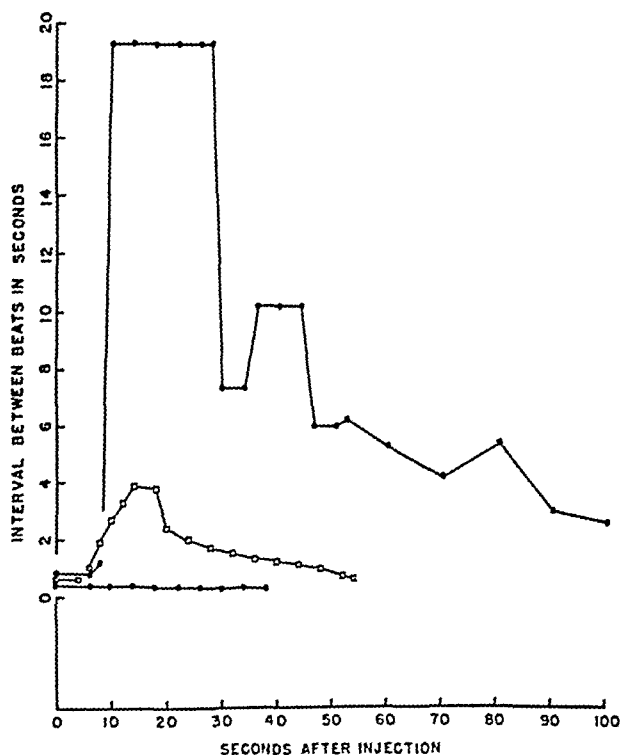


Fig. 4. January 28, 1944. Dog. Nembutal anesthesia. Elevation of serum potassium due to anuria following bilateral ligation of the ureters. Synergistic effects on heart rate of simultaneous increase in serum calcium and in serum potassium. A standard dose of 0.5 cc. of 1:5,000 acetyl- β -methylcholine chloride was given intravenously in each experiment. *Open circles*: serum potassium 8.0 m.eq. per liter, serum calcium 10.0 mgm. per cent. *Open squares*: serum potassium 13.0 m.eq. per liter, serum calcium unchanged. *Solid circles*: serum potassium 13.0 m.eq. per liter, serum calcium 38.0 mgm. per cent.

In experiments in which intravenous injection of adrenalin was employed to elicit vagal reflexes, it was observed that alteration in the level of serum potassium did not influence the general blood pressure reaction.

DISCUSSION. These experiments with dogs demonstrate a marked variation in the effectiveness of parasympathetic transmission with small changes in the concentration of potassium in serum. Excitatory actions, such as motor stimulation of the stomach, vary with concentration of serum potassium just as do inhibitory actions of the parasympathetic system. It may be recalled in this connection that

the enhancement by potassium salts of gastric contractions induced by vagal stimulation was first noted in the frog by Kupalow (16) in Babkin's laboratory. Since the level of serum potassium in the dog may vary spontaneously by as much as 3 m.eq. per liter, it is quite probable that significant variations in the responsiveness of the heart and the gastro-intestinal system may result from physiological alterations in the serum potassium.

The enhancement of the response to acetyl- β -methylcholine chloride by elevation of serum potassium suggests a common locus of action for both substances. Increased liberation of acetylcholine by vagal endings is not excluded by these experiments, but is rendered unlikely by certain recent observations of Nachmansohn and Machado (17). They found that potassium does not facilitate the formation of acetylcholine in cell-free media, although tissues do react to acetylcholine with increased vigor when small amounts of potassium are added to the perfusate. Diminution in the rate of destruction of acetylcholine by esterase is probably not the basis for the increased response to acetylcholine (18, 19) caused by potassium, so that its action differs from that of eserine and related substances. Some undefined association of potassium and acetylcholine at the surface of cells is indicated. The suggestion of Mann, Tannenbaum, and Quastel (20) that potassium increases the permeability of cells to acetylcholine is at present purely speculative.

The lack of influence of an elevated serum potassium upon the pressor response to adrenalin suggests that no reciprocal influence upon the sympathetic mechanism accompanies its effect on the parasympathetic system. There is also no indication of any antagonism between the actions of potassium and of calcium on the heart, such as occurs in cold-blooded animals. This confirms previous failure to demonstrate such consistent antagonism in the mammalian heart (21). The effects of calcium, unlike those of potassium, are demonstrable only with alterations of serum calcium considerably beyond the normal range.

It is well recognized that the parasympathetic division varies widely in the vigor of many of its manifestations, such as the carotid sinus reflex. The rôle which variations in the concentration of potassium may play in such variations of autonomic function is at present unknown. Potassium is not unique among naturally occurring substances in its stimulating action; for example, excess of thyroid hormone is also known to facilitate parasympathetic activity (22). Any modern picture of autonomic function must take into account the existence of these physiological modifying factors.

SUMMARY AND CONCLUSIONS

1. The effectiveness of vagal stimulation is reversibly enhanced by an increase in the concentration of potassium in serum.
2. This increased responsiveness to vagal stimulation is demonstrable at levels of serum potassium which are in themselves without influence on heart rate or intracardiac conduction.
3. This facilitation probably results from an increased sensitivity of the tissues to acetylcholine.

REFERENCES

- (1) HOWELL, W. H. *This Journal* 6: 181, 1901-2.
- (2) HOWELL, W. H. *This Journal* 15: 280, 1905.
- (3) HOWELL, W. H. AND W. W. DUKE. *J. Physiol.* 35: 131, 1906-7.
- (4) HERING, H. E. *Pflüg. Arch.* 161: 537, 1915.
- (5) LOEWI, O. *Pflüg. Arch.* 193: 201, 1922.
- (6) BROWN, G. L. AND W. FELDBERG. *J. Physiol.* 86: 290, 1936.
- (7) FELDBERG, W. AND G. A. GUIMARÃES. *J. Physiol.* 86: 306, 1936.
- (8) WILSON, F. T. AND S. WRIGHT. *Quart. J. Exper. Physiol.* 26: 127, 1936.
- (9) NACHMANSOHN, D. *Yale J. Biol. Med.* 12: 565, 1940.
- (10) NACHMANSOHN, D. AND B. MYERHOF. *J. Neurophysiol.* 4: 348, 1941.
- (11) NACHMANSOHN, D. AND H. B. STEINBACH. *J. Neurophysiol.* 5: 108, 1942.
- (12) NACHMANSOHN, D. AND H. B. STEINBACH. *J. Neurophysiol.* 6: 203, 1943.
- (13) HOFF, H. E., P. K. SMITH AND A. W. WINKLER. *J. Clin. Investigation* 20: 607, 1941.
- (14) WINKLER, A. W., H. E. HOFF AND P. K. SMITH. *This Journal* 124: 478, 1938.
- (15) HOFF, H. E., P. K. SMITH AND A. W. WINKLER. *This Journal* 125: 162, 1939.
- (16) KUPALOW, P. S. *Pavlov's Jubilee Volume, Leningrad-Moscow*, p. 287, 1925.
- (17) NACHMANSOHN, D. AND A. L. MACHADO. *J. Neurophysiol.* 6: 397, 1943.
- (18) NACHMANSOHN, D. *Nature* 145: 513, 1940.
- (19) MENDEL, B., D. MUNDELL AND F. STRELITZ. *Nature* 144: 479, 1939.
- (20) MANN, P. J. G., H. TANNENBAUM AND J. H. QUASTEL. *Biochem. J.* 33: 822, 1939.
- (21) WINKLER, A. W., H. E. HOFF AND P. K. SMITH. *This Journal* 127: 430, 1939.
- (22) WISE, B. AND H. E. HOFF. *J. Pharmacol. and Exper. Therap.* 64: 217, 1938.

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MODIFIED SPONTANEOUS ACTIVITY RHYTHMS IN RATS

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Temperature and light have both been demonstrated to be environmental factors responsible in part for the daily spontaneous activity rhythms of rats (Szymanski, 1918; Hemmingsen and Krarup, 1937; Browman, 1937, 1943a, 1943b). It was recently reported that modification of the normal 24 hour to a 16 hour rhythm could be induced in rats by the manipulation of artificial light (Browman, 1943a). Normal and blinded rats under constant light or dark conditions respond to controlled daily temperature changes by having their peaks of greatest activity during the daily cool period (Browman, 1943b).

Since rats can be induced to have 16 hour daily rhythms when subjected to light changes every 8 hours, and since rats also respond to daily temperature changes, would it be possible still further to modify the spontaneous activity day by control of both daily light and temperature changes? For example, could rats be induced to have 2 peaks of spontaneous activity per 24 hours instead of the usual 1?

METHODS. The methods and apparatus were the same in general as employed in previous observations on spontaneous activity (Browman, 1943a, 1943b). A Telechron time switch automatically turned fluorescent lights on or off every 6 hours. The thermostats were manually operated. Temperatures were set at 16°C. (60°F.) during the dark 6 hour periods, and 27°C. (80°F.) during the light 6 hour periods. Temperature control was possible within a range of less than 2°C. for any setting. All 8 a.m., 2 p.m. and 8 p.m. readings and settings were done by the author. All 2 a.m. readings and thermostat adjustments were made by a conscientious night watchman. Control of minimum humidity (45 per cent) only was possible with available apparatus.

Since only 16 voluntary work registering cages of the revolving type were available it was necessary to run 2 series of experiments consecutively. Both series of animals came from a basic 16 generation inbred line. The first series represented animals coming from a sub-strain which had been maintained for 5 generations in constant dark. The second series consisted of animals from another substrain maintained for 5 generations under conditions of continuous light. All experimental animals were born in the artificial 12 hour day, and all

enucleated animals had both eyeballs removed on the day of birth. The rats were weaned at 28 days of age, and immediately placed in activity cages. Recordings of spontaneous activity began at 40 days of age. Animals that were spayed at 70 days of age were lightly etherized during a warm light 6 hour period, ovariectomized via a ventral abdominal incision, and promptly returned to the activity age.

The phrase "a 24 hour activity rhythm" in this report means that the peak of daily spontaneous activity is confined to either one or the other of the 2 dark cold periods of each 24 hour day as set up in this investigation. The term "12 hour activity rhythm" refers to the fact that the peaks of daily activity are approximately equally distributed between the 2 dark cold periods of each 24 hour day. An animal is said to have shifted from one type of rhythm to another only when the new rhythm has continued for at least 4 days (see fig. 1).

TABLE 1

Spontaneous activity of normal and blinded female rats in an artificial 12 hour day

SERIES	NO. OF RATS	CONDIT. OF EYES	FIRST 30 DAY PERIOD		TREATMENT AT END OF 1ST PERIOD	SECOND 30 DAY PERIOD	
			Average daily activity	Average no. da. in 12 hr. rhythm		Average daily activity	Average no. da. in 12 hr. rhythm
I	7	Nor.	2,725	9.8	None	3,962	.7
	9	Bl.	5,319	8.0	None	7,815	0.0
II	4	Nor.	7,395	7.3	None	12,392	4.5
	4	Nor.	8,175	10.0	Spayed	2,993	11.7
	4	Bl.	12,604	10.2	None	17,889	8.2
	4	Bl.	11,235	17.2	Spayed	5,135	16.5

RESULTS. The averages of the activity of all 16 animals in series II for the 4 different 6 hour periods per day result in a clear cut bimodal curve, with the two peaks of activity in the dark cold and the low points in the light warm periods. The same bimodal curve results in each case where the daily activities of the 4 categories of experimental animals, i.e., normal and blind unsplayed, and normal and blind spayed, are averaged. The average activity of each of the four 6 hour periods also results in sharp bimodal curves in the cases of 7 individual rats. If no further analysis were made the incorrect assumption would be that the experimental conditions had induced 12 hour rhythms in a significant number of animals during most of the period under observation.

Examination of the daily records and graphs of daily activity however reveals that some animals have 24 hour rhythms with daily peaks of activity in one dark period, e.g., 2 p.m. to 8 p.m., and then suddenly shift and have the daily stint of activity in the other dark period, e.g., 2 a.m. to 8 a.m. The average of these total daily activities for a given period yields a bimodal curve. It is true that most of the animals exhibiting the type of activity mentioned above also had varying periods of time during which 12 hour rhythms actually did occur (table 1).

Table 1 indicates the average duration of 12 hour rhythms in each of the several experimental categories. Only 2 animals out of both series persisted in 24 hour rhythms for 60 days, and 2 in the 12 hour rhythm for as long as 33 and 37 days. The usual type of daily activity pattern was a 24 hour rhythm

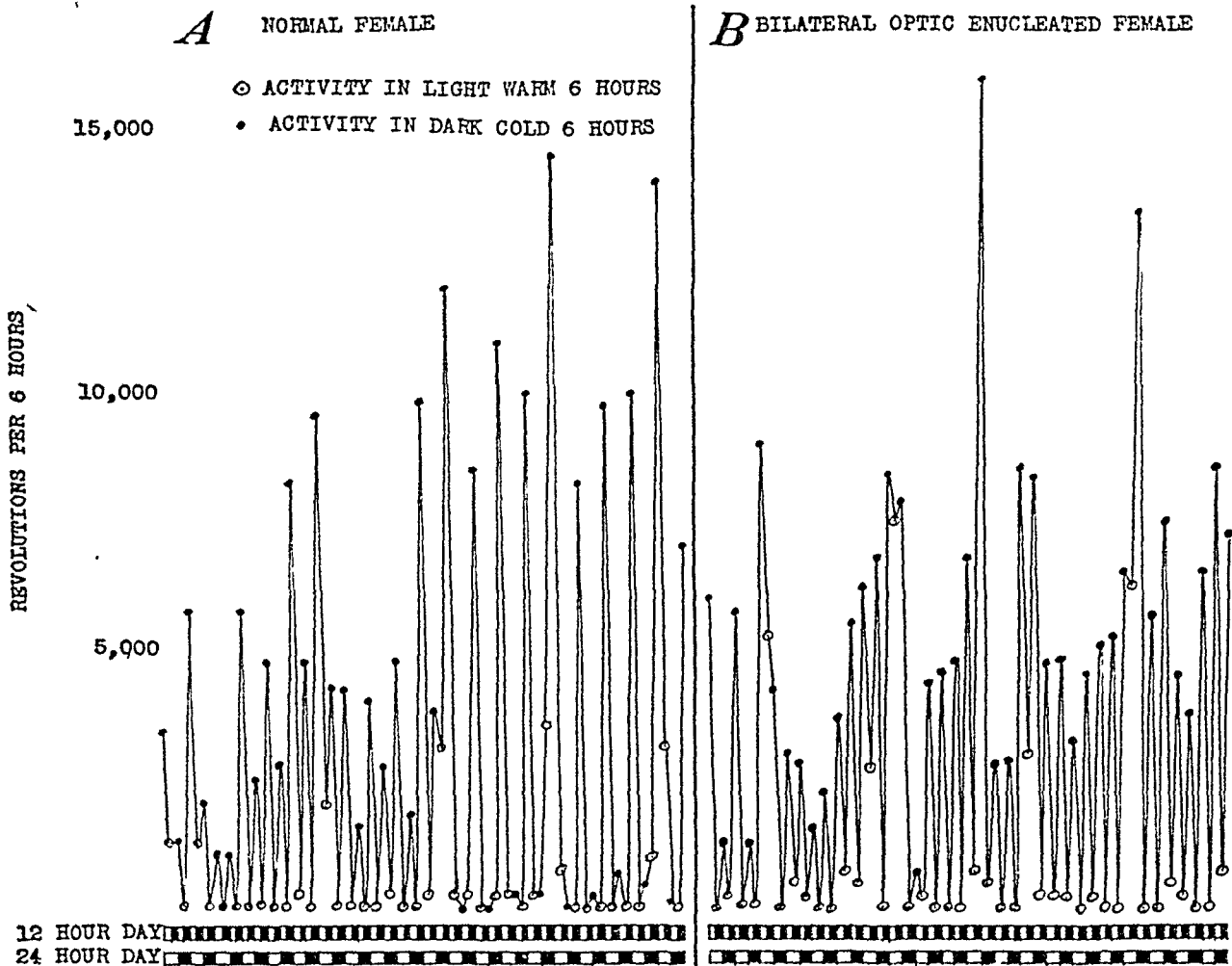


Fig. 1. Black dots (●) indicate the total number of revolutions during the dark cold 6 hour intervals, i.e., 2 a.m.-8 a.m. and 2 p.m.-8 p.m. The circumscribed dots (○) indicate the spontaneous activity during the warm lighted 6 hour intervals, i.e., 8 a.m.-2 p.m., and 8 p.m.-2 a.m.

Fig. 1A. Normal female; illustrates 10 days of 12 hour activity rhythm with a sudden shift to the 24 hour activity rhythm for 10 days.

Fig. 1B. Bilateral optic enucleated female; illustrates 20 days of a 12 hour activity rhythm.

interrupted by a 12 hour rhythm of varying duration. Figure 1A shows a normal unspayed female having a 12 hour rhythm, and then shifting abruptly to a 24 hour rhythm. Rats can be induced to have 12 hour spontaneous activity rhythms for varying periods of time by the use of controlled temperatures and light.

Figure 1B represents a graph of spontaneous activity characteristic of animals considered to be in a 12 hour rhythm. Figures 1A and 1B represent the activities of normal eyed and bilateral optic enucleated litter mate females running in adjacent cages from June 2 to June 22. No consistency was observed in the times of shift, or duration of activity rhythms of either pattern, in any litter-mates.

Normal and blinded animals in series I shifted their patterns of daily rhythm approximately the same number of times. Normal animals averaged 2.2 (range 1-5), and the blind 2.1 (range 1-3) shifts in activity. Casual inspection would suggest that spayed animals persisted in 12 hour rhythms longer than non-spayed females, but actually when compared to their own activities before spaying the spayed animals persisted with essentially the same average.

Blind animals in both series were consistently more active than normal eyed animals (Browman, 1942), and in series II persisted in 12 hour rhythms significantly longer than normal eyed animals. The normal eyed animals had special senses intact for receiving both light and temperature stimuli, whereas blinded animals presumably could only be influenced by temperature changes.

The difference in activity and rhythms between the two substrains, one from a constant dark line and the other from a continuous light line, may be largely genetic factors rather than purely a reflection of the difference in environmental backgrounds.

Vaginal introitus had no marked influence on the daily rhythm, for 12 of 16 females did not shift the daily rhythm immediately before, during, or immediately after vaginal introitus. Three only of the 12 were in the 12 hour rhythm at introitus. These three continued in this rhythm for at least 10 days before shifting rhythms. Of the 9 in 24 hour rhythms the earliest shift to the 12 hour rhythm occurred 7 days after introitus. The 4 females which did shift rhythms immediately after introitus did so during, or within $1\frac{1}{2}$ days of, vaginal introitus. Three of the 4 shifted from a 24 hour to a 12 hour rhythm, and the other shifted from a 12 hour rhythm to a 24 hour rhythm.

The influence of the oestrous cycle can be seen in the spurts of activity occurring every 4 to 5 days (fig. 1A and 1B). Inspection of other similar graphs suggests that 6 hours may not be sufficient time for the female in oestrus to "run herself out." Vaginal smears were taken of 6 females for 3 weeks, during which time 3 females shifted rhythms, and the shifts happened to occur during proestrus in each case.

The gradual increase in spontaneous activity in this strain of rats as they grow older, reaching a peak at about three months of age, has been previously reported (Browman, 1942). This difference in activity due to age can be seen in table 1 where an increase of 60 to 70 per cent during the 2nd 30 day period occurred in the non-spayed animals. Simultaneously activity of spayed litter-mate females decreased. Although spaying reduced the total spontaneous activity of both normal eyed and blinded females it had no significant influence on the average number of days of either group in the 12 hour rhythm (table 1). Rats up to 70 days of age seemed to have a slightly greater number of days in the 12 hour rhythm than they did following day 70.

SUMMARY

Thirty-two rats of an inbred strain were subjected to an artificial day length of 6 hours of light at 27°C., and 6 hours of dark at 16°C. for approximately 2 months. Although no animal maintained a consistent 12 hour rhythm for more than 37 days, 30 of 32 rats did exhibit 12 hour rhythms for varying lengths of time. Spaying animals at the end of the 1st month of readings had no marked influence on the type of daily rhythm, although total daily activity was less. Rats can be induced to have 12 hour spontaneous activity rhythms, i.e., 2 peaks of activity per 24 hour day, for varying lengths of time by controlling both temperature and light conditions.

REFERENCES

- BROWMAN, L. G. J. *Exper. Zool.* **75**: 375, 1937; **91**: 331, 1942; **94**: 477, 1943b; *J. Comp. Psych.* **36**: 33, 1943a.
HEMMINGSSEN, A. M. AND N. B. KRARUP. *Det Kgl. Danske Videnska-bernes Selskab. Biol. Meddel.* **13**: 1, 1937.
SZYMANSKI, J. S. *Pflüger's Arch.* **171**: 324, 1918.

THE RELATION OF ADRENALIN AND OF THE CAROTID SINUS TO THE HYPERGLYCEMIA OF SHOCK

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The hyperglycemia of shock has received increasing consideration as problems of metabolism related to this clinical picture have assumed greater importance (1, 2). The earlier view (3, 4) associating it with vasoconstriction as a response to increased adrenalin discharge left many unsolved problems. The two phenomena bear no constant relation to each other either in their time of appearance or in the degree of their development. In traumatic shock vasoconstriction becomes evident promptly and is severe while hyperglycemia is late and mild. After hemorrhage their occurrence relates itself to the speed of the loss of blood. When this is reasonably rapid the hyperglycemia attains a height much greater than occurs in traumatic shock, the vasoconstriction also is marked but delayed in its appearance (5). Variations like these as well as the comparatively mild hyperglycemic response to adrenalin infusion when compared to the response to hemorrhage have led to study of other possible mechanisms that may be involved.

Attention has been directed to factors concerned with variations in blood sugar levels. These have included the influence of rapid decrease in blood volume. The results of this study led to investigation of possible involvement of receptors in the vascular bed and their response both to rapid change in blood volume and to increased adrenalin blood content.

Hyperglycemia in traumatic shock. The method chosen for production of traumatic shock in dogs was the tight application of tourniquets to both hind legs for five hours. The changes following release have been detailed in earlier publications (6). In four animals studied for this purpose the maximal and ultimate rise in blood sugar was attained within an hour after tourniquet release when it was 153, 159, 167 and 176 mgm. per cent respectively.

Hyperglycemia and its relation to rate of blood loss. The rise in blood sugar after hemorrhage first observed by Claude Bernard (7) has been confirmed repeatedly. Much of the recent work has been related to shock and there are available, as a consequence, levels of blood sugar associated with different rates of blood removal. It is difficult to compare these as the investigations were not directed primarily to the problem and many variables including animal species are concerned. However, analysis of recent reports shows clearly that in rats the hyperglycemic response is slight or absent when the withdrawal of blood is

¹ The work described in this paper was done with the aid of grants from the Commonwealth Fund and under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Yale University.

slow and much more marked when it is rapid (2). This was confirmed independently with many dogs; the experiments that follow are typical.

Prevention of hyperglycemia by prompt restoration of blood volume after hemorrhage. The discrepancy between the hyperglycemic response to slow and rapid

TABLE 1

Dog 1

Rapid bleeding. Blood amounting to 4% of the body weight was withdrawn in 80 minutes. The venous blood sugar reached 526 mgm. %.

TIME	BLOOD WITHDRAWN	VENOUS SUGAR
<i>min.</i>	<i>% of body weight</i>	<i>mgm. %</i>
0	1.0	113
20	2.0	113
40	3.0	138
60	3.5	
80	4.0	228
107	4.25	
120	4.5	
135	4.75	
140		500
150	5.0	526

TABLE 2

Dog 2

Slow bleeding. Blood amounting to 4% of the body weight was withdrawn in 255 minutes. The venous blood sugar reached only 162 mgm. %.

TIME	BLOOD WITHDRAWN	VENOUS SUGAR
<i>min.</i>	<i>% of body weight</i>	<i>mgm. %</i>
0	1.0	93
60	2.0	
90	2.25	115
120	2.5	
165	3.0	
210	3.5	119
255	4.0	
315	4.5	137
345	5.0	
375	5.5	146
420		162

bleeding suggested that this might be related to time required for the passage of fluid into the blood vessels from the tissues (8). To put this to test, effort was made to keep the blood volume constant by the replacement of withdrawn blood with Tyrode solution immediately after each bleeding. Under these circumstances and even when the withdrawal of blood is graded in accord with a maximal increase in blood sugar, such replacement with Tyrode solution results in no

change from normal in the blood sugar level. It should be noted that the usual marked vasoconstriction associated with this rate of blood withdrawal does not occur. If it appears at all it is a mild and terminal event. The following figures from one of many experiments are typical. With the withdrawal of blood in amounts of 1 per cent of body weight every twenty minutes for the first hour, and $\frac{1}{2}$ per cent every twenty minutes thereafter, the following figures were obtained: before bleeding 100 mgm. per cent; at 2 per cent, 104 mgm. per cent; at 4 per cent, 102 mgm. per cent; at 6.5 per cent, 103 mgm. per cent; at 7.5 per cent, 107 mgm. per cent blood sugar.

The relation of anoxia to hyperglycemia. It will be recalled that slow removal of blood only results in a mild hyperglycemia, and that even this slight response is eliminated when replacement with equal quantities of Tyrode solution promptly follows each withdrawal. In both of these circumstances the red blood cells are ultimately greatly reduced and as the jugular venous oxygen approaches 1 volume per cent or even less the animals die. Even with such extreme anoxia the blood sugar tends to remain constant.

The influence of receptors in the vascular bed on the hyperglycemia of hemorrhage. Since the hyperglycemia of hemorrhage cannot be ascribed to anoxia, but is associated with rapid diminution in blood volume and can be prevented when this is kept constant, the possibility was considered that receptors in the vascular bed might be involved in its excitation. Several series of experiments were carried out in this exploration. The first included six dogs subjected to carotid sinus ligation² immediately before bleeding; the second, an equal number of animals whose carotid sinuses had been similarly ligated a week before the hyperglycemic response to hemorrhage was tested; the third and fourth involved immediate and preliminary stripping of the sheaths of the sinuses and of the adjacent common, internal and external carotid arteries; the fifth and sixth were concerned with ligation of the vertebral arteries and subsidiary branches from the sub-clavian just before or a week before bleeding. These latter two series were undertaken as control, to determine the relative importance of occlusion of other large supply vessels to the brain, as compared to influence of blood volume upon vascular receptors.

None of these procedures had any influence upon the hyperglycemic response to hemorrhage.

The hyperglycemic response to adrenalin. The demonstration of increased adrenalin discharge (11, 3) after hemorrhage has been considered as the cause of the hyperglycemic response, even though there is not unanimity of opinion concerning either the mechanism involved in the increased adrenalin discharge or how this raises the blood sugar level (12, 13).

The normal dog responds to a single intravenous injection of adrenalin amounting to 0.02 mgm./kgm. or even of 0.07 mgm./kgm. with a gradual rise in blood sugar that reaches its maximum of only approximately 60 mgm. per cent in an

² After the common carotid is freed from its fascial sheath it is ligated $\frac{3}{4}$ inch below the bifurcation; the internal and external carotids are freed and ligated separately $\frac{1}{2}$ inch distal to the bifurcation, and the sinus is then opened and emptied of blood (9) (10).

hour or less. A very similar result follows if the same quantity of adrenalin in fifteen cubic centimeters of normal saline is infused intravenously and no difference has been noted whether the time of infusion was five minutes or fifteen³. These results in the dog, concerning the rise in blood sugar level following adrenalin, are in accord with those of DeBodo (14).

When the adrenalin infusion is continued for an hour or even for 90 minutes and when the total amount of adrenalin infused is as large as 0.18 mgm./kgm., the hyperglycemic response is only slightly greater. The height of the rise in blood sugar, interestingly enough, occurs during the course of the infusion, frequently within the first hour and even though the infusion is continued at the same rate the blood sugar often begins to decline. Similar findings are included by Cori et al. (12) in a table of seven dogs infused with adrenalin for three hours, and the observations of Griffith et al. (15) are also in accordance with these results.

This limited response to adrenalin contrasts sharply with the marked blood sugar change after hemorrhage. There is a further difference. While the starved dog with little glycogen in its liver does not react to adrenalin as much as the well fed animal, the rise in blood sugar in the latter is only slightly greater. The rise in blood sugar after hemorrhage, on the other hand, is materially influenced by the glycogen content of the liver. This becomes evident from a series of experiments that may be summarized as follows: The blood sugar reached 280 to 340 mgm. per cent after standardized rapid bleeding of dogs on a normal diet; when this was supplemented a day before bleeding by 50 to 60 grams of dextrose, the sugar of the blood reached from 430 to 550 mgm. per cent as determined in five animals.

The influence of receptors in the vascular bed on the hyperglycemic response to adrenalin. With the evidence that the known receptors in the vascular bed are not involved in the blood sugar response to hemorrhage and the extensive but still inconclusive investigations implicating the nervous system in the adrenalin response, a series of experiments were undertaken quite similar to those included above under hemorrhage. They included injection or infusion of adrenalin either immediately or a week after ligation of the carotid sinuses, after immediate or preliminary stripping of the sinuses without narrowing the vascular lumina and after immediate or preliminary ligation of the vertebral arteries.

Sinus ligation either eliminates or greatly modifies the hyperglycemic response to injection or infusion of adrenalin in quantities of 0.02 to 0.05 mgm./kgm. during 5 to 15 minutes. When there is a response it contrasts markedly with that of the normal dog. The slow rise of approximately 60 mgm. per cent followed by an equally slow decline, both together extending over an hour or more, becomes a sharp rise not exceeding 30 to 40 mgm. per cent, reaching its apex in 10 to 15 minutes and returning to normal within an equal period. This sharp rise and fall were found more frequently when the animals' regular diet had been supplemented with 50 to 60 grams of dextrose on the day preceding test.

³ Whenever the length of the infusion period exceeded five minutes, precautions were taken to prevent adrenalin oxidation by keeping the infusion fluid slightly acid.

Animals that showed no rise in blood sugar after adrenalin injection were bled several hours later without having been fed in the interim and then showed the usual marked and prolonged hyperglycemic response. In this way and also by determination of liver glycogen after injection or infusion of adrenalin, it was clearly demonstrated that lack of the hyperglycemic response to adrenalin in sinus ligated dogs follows even when the liver contains available glycogen that is discharged on bleeding. The example that follows illustrates several of these findings.

Qualitatively similar changes in adrenalin response follow immediate or preliminary stripping of the sinuses or ligation of the vertebral arteries. Complete elimination of the rise in blood sugar is less frequent. The short sharp rise and return to pre-injection level is more common.

TABLE 3

Dog 3

If the carotid sinuses are ligated, adrenalin hyperglycemia is small and of short duration. In contrast to this, the hyperglycemia following bleeding is undiminished.

DATE	TIME	BLOOD WITHDRAWN	VENOUS SUGAR
		% body weight	mgm. %
6/12/44	9:00 a.m. Injection of 0.035 mgm./kgm. adrenalin		113
	9:05		114
	9:15		145
	9:25		125
	9:35		106
	9:45		100
	10:00		103
6/12/44	2:00 p.m.	1.0	103
	2:20	2.0	110
	2:54	2.5	188
	3:19	3.0	368
	3:29	No further bleeding	436

These results indicate that the hyperglycemia following adrenalin injection is mediated through the carotid sinus and similar neural mechanisms related to other large arterial blood sources to the head, as for example the vertebral arteries. Since these structures are not concerned in the hyperglycemic response to hemorrhage as is indicated by the experiments cited earlier, the conclusion seems proper that the mechanisms concerned in the hyperglycemia associated with the two procedures are not the same. It becomes doubtful whether increased adrenalin discharge, demonstrated to occur with rapid blood withdrawal, is the only or decisive factor in the associated hyperglycemia.

Insulin sensitivity after carotid sinus denervation or vertebral artery ligation. As a possible aid in determining the rôle of the carotid sinus mechanism in adrenalin hyperglycemia the fact that adrenalin is necessary for restoration of blood sugar

after insulin hypoglycemia (16) was used as a point of departure. When adrenalin is lacking, return of blood sugar to normal values is either greatly retarded or impossible. This is expressed in an increased insulin sensitivity as occurs experimentally after adrenal demedullation. With this background insulin sensitivity was studied in animals that did not respond to adrenalin with hyperglycemia after carotid sinus or vertebral artery ligation.

Ten animals were included in the series. In six the sinuses were ligated, the vertebral arteries in the other four. After preliminary determination of insulin sensitivity, the operations were completed and three to seven days were allowed to elapse before the insulin reaction was again tested. The example that follows only differs from the group inasmuch as the clinical signs were more striking.

TABLE 4

Dog 4

Prolonged and intensified effect of insulin after carotid sinus ligation

DATE	TIME IN MIN.	BLOOD SUGAR	REMARKS
		<i>mgm. %</i>	
6/17/44		82	Weight 11.4 kgm. 8 U insulin intramuscularly
	30	70	Restless
	60	57	
	80	48	
	100	46	Weak, lying down
	120	52	
	140	61	Still quiet
6/19/44			Bilateral carotid sinus ligation
6/22/44		93	Weight 11.4 kgm. 8 U insulin intramuscularly
	30	77	Agitated, restless
	60	66	
	80	58	Unsteady, quiet
	100	43	Unable to stand
	120	44	Convulsion; 1 gram glucose intravenously
	140	47	
	230	65	Convulsion

The increased sensitivity to insulin was common to all ten dogs as expressed by the protracted hypoglycemia and the slow return of the blood sugar to normal.

The results secured with insulin administered intramuscularly were corroborated in detail with another group of animals that received insulin intravenously. It should be noted that larger quantities given in broken doses at short intervals are required for production of the same degree of hypoglycemia on intravenous as compared with intramuscular injection. This is in accord with the findings of Maxwell and Bischoff (17), that retardation of insulin absorption by addition of ferric salts greatly enhances the activity.

DISCUSSION. Evidence has been presented indicating that the hyperglycemia after hemorrhage and that associated with adrenalin infusion involve different

mechanisms. Blood volume would seem to be of primary importance in the former, but it is not clear how this operates. Hyperglycemia after hemorrhage does not result from anoxia; it does not depend upon the carotid sinus or upon similar, heretofore unsuspected mechanisms in the vertebral arteries. Without doubt the sugar is derived from the liver, but there is no clear indication how the increased discharge into the blood after hemorrhage is brought about.

The very high values regularly encountered, even when there is spilling of blood sugar into the urine, contrast sharply with the comparatively mild hyperglycemia of traumatic shock and after adrenalin infusion. This is an expression of increased sugar release by the liver irrespective of its derivation from pre-formed glycogen, protein or lactic acid. It may be a manifestation of early regressive hepatic cell change resulting from some definite cause, perhaps related to stagnant anoxemia, and more harmful than anoxia alone. Analogy for this is the unlimited escape from cells with impaired function of substances normally held in their interior. An extreme example is the loss of potassium and of phosphates from muscle after release of tourniquets.

The mild hyperglycemic response to adrenalin even when this is in great excess, suggests that adrenalin functions in association with a controlling mechanism designed to limit its effect. This statement is based upon experience with the living animal. Experiments included in the foregoing report leave no doubt that adrenalin hyperglycemia is mediated through the carotid sinuses and similar mechanisms of the vertebral arteries. These facts do not preclude a more direct and perhaps less controlled action of adrenalin on glycogen cleavage and sugar discharge from the liver in perfusion experiments, when all but local neural control is eliminated. They obviously bear no relation to possible influence of adrenalin on enzyme action (18) in isolated systems or with tissue slices.

With the information at hand that the carotid sinus and comparable mechanism of the vertebral artery are involved in adrenalin hyperglycemia it became desirable to scrutinize explanations offered in the literature for similar variation of adrenalin hyperglycemia in clinical and experimental conditions. Many of these involve the hypophysis. As early as 1912 Aschner (19) showed that hypophysectomized animals did not respond to adrenalin injection with glycosuria. This was later corroborated by several investigators from the standpoint of the blood sugar level (20, 21). More recently DeBodo and his associates (14) have studied the question intensively. Their experiments show conclusively that hypophysectomy minimizes or eliminates the hyperglycemic response to adrenalin. Moreover, they have demonstrated that this occurs even when adequate glycogen is present in the liver. These experiments establish the relation of hypophysectomy to the hyperglycemic adrenalin response, but the detail of how this mechanism operates is undetermined. This is stated clearly in the following quotation. "Thus, we are forced to conclude that there is definite impairment in the mobilization of the glycogen in response to infused adrenalin in the absence of the hypophysis (14)."

These experimental findings are in accord with recognized clinical conditions, in which the hyperglycemic response to adrenalin may be eliminated—Cushing's disease is an example.

When these observations are reviewed in the light of the demonstrated relation of the carotid sinus and the mechanisms of the vertebral artery to adrenalin hyperglycemia, it becomes desirable to bear in mind the intimate relations between the hypophysis and neural structures (22-24), including those in its stalk and fibers from the Vidian ganglion.⁴ Hypophysectomy obviously destroys these relations (25) as may also occur when the gland is involved in morbid change. This raises doubt whether the hypophysis as such is involved in the lack of the hyperglycemic response to adrenalin.

The glandular activity itself as distinct from the neural mechanisms is better differentiated for insulin hypersensitivity. Following the demonstration of increased sensitivity by Houssay in 1925 (26) and its repeated confirmation (20, 21), it has come to be a test for the completeness of removal of the gland. The later discovery of factors derived from the hypophysis governing glyconeogenesis including adrenotropic and diabetogenic hormones, provided essential knowledge for the understanding of the alteration in carbohydrate metabolism after hypophysectomy.

The mild insulin hypersensitivity after carotid sinus or vertebral artery ligation is undoubtedly in part caused by the failure of adrenalin to restore the blood sugar level to normal. Insulin hypersensitivity after extirpation of the Vidian ganglion as demonstrated by Zacharias (27, 28) well may result from the interference with the same mechanism upon which adrenalin is dependent. Zacharias ascribed insulin hypersensitivity after Vidian ganglion extirpation to reduction of hypophyseal function, an explanation complicated by her observation that the gonadotropic function of the hypophysis was increased by the same procedure. Whether the insulin hypersensitivity after Vidian ganglion extirpation was of the order of magnitude observed after hypophysectomy or only the milder grade resulting after carotid sinus denervation or vertebral artery ligation cannot be concluded from her publication.

The greater incidence of insulin convulsions found by Zacharias may have a different explanation. Gellhorn (29) observed that the intensity and frequency of convulsions following injection of metrazol, etc., are increased by the inactivation of the carotid sinuses. If it is assumed that extirpation of the Vidian ganglion interrupts pathways leading from the carotid sinuses to the central nervous system, increase in intensity or frequency of convulsions would be expected.

The findings reported above in table 4, typical for a group of several animals, suggest that carotid sinus ligation increases the susceptibility to convulsions after insulin.

⁴ The small mass of ganglion cells at the origin of the Vidian nerve, i.e., at the junction of the greater superficial petrosal nerve and the great deep petrosal nerve, was indicated as the Vidian ganglion by Zacharias (28).

This evidence together with Gellhorn's observations indicates that insulin hypersensitivity after hypophysectomy is dependent both upon removal of the hormonal influences of the gland and upon the neural mechanism essential for adrenalin actions for the mobilization of sugar and the response of the central nervous system to stimuli.

SUMMARY

1. Hyperglycemia after hemorrhage varies in extent with the rate of blood loss. It can be prevented by quantitative replacement with Tyrode solution immediately after each blood withdrawal.

2. The anoxia attained is independent of blood loss or of replacement with Tyrode solution and cannot be the cause of the elevated blood sugar.

3. Carotid sinus or vertebral artery ligation does not influence hyperglycemia after hemorrhage.

4. Adrenalin hyperglycemia is mild as compared with that of hemorrhage.

5. Carotid sinus or vertebral artery ligation abolishes or minimizes the hyperglycemic response to adrenalin even when there is adequate liver glycogen as demonstrated by chemical assay or by response to bleeding.

6. The dependence of adrenalin hyperglycemia upon this neural mechanism suggests that the lack of the hyperglycemic response to adrenalin after hypophysectomy may be a result of damage to this pathway rather than to absence of the gland.

7. Insulin sensitivity also is increased after carotid sinus or vertebral artery ligation, but not to the extent that follows hypophysectomy. It may result from interference with adrenalin activity essential both for the restoration of blood sugar and for the decrease of the excitability of the central nervous system.

REFERENCES

- (1) GOVIER, W. M. AND C. M. GREER. *J. Pharmacol. and Exper. Therap.* **72**: 317, 1941.
- (2) ENGEL, F. L., M. G. WINTON AND C. N. H. LONG. *J. Exper. Med.* **77**: 397, 1943.
- (3) SAITO, S., B. KAMEI AND H. TACHE. *Tohoku J. Exper. Med.* **2**: 205, 1928.
- (4) BROOKS, C. M. *This Journal* **114**: 30, 1935-36.
- (5) MYLON, E., C. W. CASHMAN, JR. AND M. C. WINTERITZ. *This Journal* **142**: 299, 1944.
- (6) MYLON, E., M. C. WINTERITZ AND G. J. DESÜTÖ-NAGY. *This Journal* **139**: 313, 1943.
- (7) BERNARD, C. *Leçons sur le diabète*. Paris, p. 210, 1877.
- (8) ROBERTSON, J. D. *J. Physiol.* **84**: 393, 1935.
- (9) GOLLWITZER-MEYER, K. AND H. SCHULTE. *Arch. f. exper. Path. und Pharmakol.* **165**: 685, 1932.
- (10) HEYMANN, C., J. J. BOUCKAERT AND P. REGNIERS. *Le sinus carotidien*. G. Doin & Co., Paris, 1933.
- (11) BEDFORD, E. A. AND H. C. JACKSON. *Proc. Soc. Exper. Biol. and Med.* **13**: 85, 1916.
- (12) CORI, C. F., R. E. FISHER AND S. T. CORI. *This Journal* **114**: 53, 1935-36.
- (13) HIMSWORTH, H. P. AND D. B. MCN. SCOTT. *J. Physiol.* **93**: 159, 1938.
- (14) DEBODO, R. C., H. I. BLOCK AND I. H. GROSS. *This Journal* **137**: 124, 1942.
- (15) GRIFFITH, F. R., JR., J. E. LOCKWOOD AND F. E. EMERY. *This Journal* **126**: 299, 1939.
- (16) CANNON, W. B., N. A. McIVER AND S. W. BLISS. *This Journal* **69**: 46, 1924.
- (17) MAXWELL, L. C. AND F. BISCHOFF. *This Journal* **112**: 172, 1935.
- (18) LEE, M. AND D. RICHTER. *Bioch. J.* **34**: 551, 1940.

- (19) ASCHNER, B. *Pflüger's Arch.* **196**: 1, 1912.
- (20) CORKILL, A. B., H. P. MARKS AND W. E. WHITE. *J. Physiol.* **80**: 193, 1933-34.
- (21) COPE, O. AND H. P. MARKS. *J. Physiol.* **83**: 157, 1934.
- (22) The hypothalamus and central levels of autonomic function. Chapters V, VI, VIII, XX. Baltimore, The Williams & Wilkins Co.
- (23) BROOKS, C. M. *This Journal* **121**: 157, 1938.
- (24) INGRAM, W. R. AND R. H. BARRIS. *This Journal* **114**: 562, 1935-36.
- (25) CHAIKOFF, I. L., F. L. REICHERT, P. S. LARSON AND M. E. MATHES. *This Journal* **112**: 493, 1935.
- (26) HOUSSAY, B. A. AND M. A. MAGENTA. *C. R. Soc. Biol.* **92**: 822, 1925.
- (27) ZACHARIAS, L. R. *Endocrinology* **31**: 638, 1942.
- (28) ZACHARIAS, L. R. *J. Comp. Neurology* **74**: 421, 1941.
- (29) GELLHORN, E., L. YESINICK, M. KESSLER AND H. HAILMAN. *This Journal* **137**: 396, 1942.

THE RENAL REABSORPTIVE MECHANISM FOR INORGANIC PHOSPHATE IN NORMAL AND ACIDOTIC DOGS

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Phosphate plays a rôle in a number of body functions: calcium phosphate is a prominent constituent of skeletal structures; organic phosphate complexes are of importance in energy transformations within cells; and, as the chief buffer component of the urine, inorganic phosphate aids the body in balancing its acid base requirements. Since the urine is the major portal of exit for excess phosphate of dietary origin, the renal mechanisms controlling loss or conservation of the body's stores of inorganic phosphate are of prime interest.

Recent investigators (2, 7, 12, 19, 22) concede that phosphate is filtered through the glomeruli, a part is reabsorbed by the renal tubules and the excess is excreted. Upon the characteristics of the reabsorptive process, which fundamentally determine the balance between conservation and elimination, there is less agreement. Harrison and Harrison (7) claim that the capacity of the renal tubules to reabsorb phosphate is limited in the same sense as Shannon and Fisher (16) had previously shown glucose reabsorption to be limited. Thus, if phosphate is presented to the renal tubules in the glomerular filtrate in amounts exceeding their capacity to reabsorb, the excess appears in the urine. The limited range of plasma phosphate concentration in their studies, i.e., from 3 to 10 mgm. of phosphate phosphorus per 100 cc., is insufficient to establish their conclusion definitely.

Smith, Ollayos and Winkler (19) reinvestigated the problem and arrived at the opposite conclusion, namely: that the capacity of the tubules to reabsorb phosphate increases progressively with increasing plasma concentration over a range from 3 to 40 mgm. per 100 cc. Their experiments were complicated by rapid falls in plasma concentration and by sharply declining rates of glomerular filtration. Coupled with their few experiments, these factors render their conclusion unconvincing.

As a preliminary to a study of the renal mechanisms for the regulation of acid base balance, we have found it necessary to determine the properties of the renal tubular reabsorptive mechanism for phosphate. Our results indicate that the capacity of the renal tubules to reabsorb inorganic phosphate is limited and that the mechanism is relatively stable and unaffected by considerable shifts in electrolyte and acid base balance of the organism.

METHODS. Our experiments have been carried out on 5 trained female dogs loosely restrained on a comfortable animal board. Water, in amounts of 50 cc. per kilo, was administered by stomach tube at the start of the experiment to ensure adequate hydration of the animal. Urines were collected by catheter and the bladder washed out with distilled water at the end of each 10 minute

urine collection period. Urine flows were maintained high to avoid dead space errors. Bloods were drawn without stasis at the midpoint of each urine collection period either from the jugular vein or from a retention needle in the femoral artery. Since no arteriovenous difference for phosphate has been observed at any plasma level, arterial and venous bloods have been used interchangeably. Continuous intravenous infusions have been administered throughout the experiments at 5, 7.5 or 10 cc. per minute through the saphenous vein. Creatinine, for the measurement of glomerular filtration rate, has been incorporated in the infusions in amounts sufficient to maintain plasma concentrations of approximately 40 mgm. per cent. A 0.5 molar solution of di- and monobasic sodium phosphate (pH 7.4) was added to the infusions in increasing amounts to attain the desired plasma concentrations. Each new infusion was administered for 15 to 20 minutes before beginning the experimental periods to establish approximate equilibrium and to minimize changes in plasma concentration during these periods. The infusions containing small amounts of phosphate were brought to approximate isotonicity by addition of glucose or sodium chloride. As will be evident later, sodium chloride is to be preferred.

Colorimetric analyses of creatinine, phosphate and glucose were performed with a photoelectric colorimeter using appropriate filters. The Folin and Wu method (5) for creatinine was applied to iron filtrates of plasma (20) and to diluted urines, with the precaution of making each colorimeter reading exactly 10 minutes after the addition of alkaline picrate. Phosphate was determined on trichloroacetic acid filtrates by a modification (12) of the method of Fiske and Subbarow (3). Chloride was determined by the method of Schales and Schales (14) as modified by Summerson (21), and glucose by the Folin method (4) as modified by Shannon, Farber and Troast (17).

RESULTS. *The determination of the maximum tubular reabsorptive capacity for phosphate.* We have quantitated the amount of phosphate reabsorbed by the renal tubules in a minute's time as the difference between the quantity filtered through the glomeruli and the quantity excreted in the urine. The quantity filtered is the product of the number of cubic centimeters of plasma filtered through the glomeruli each minute, as determined by the creatinine clearance, and the quantity of phosphate contained in each cubic centimeter of this plasma. The quantity excreted is the product of the number of cubic centimeters of urine formed each minute and the quantity of phosphate contained in each cubic centimeter of this urine. All phosphate values are expressed in terms of milligrams of phosphate phosphorus per 100 cc. or per minute.

Such an analysis implies that inorganic phosphate is freely filterable from the plasma. In the amphibian kidney this has been shown to be true by Walker and Hudson (22) by comparing phosphate concentrations in plasma and in fluid drawn from the glomerular capsule. Free filtration from mammalian plasma through collodion membranes has been demonstrated by Smith, Ollayos and Winkler (19), by Fay, Behrmann and Buck (2) and by unpublished results obtained a number of years ago by the senior author. The finding of Harrison and Harrison (7) of appreciable binding remains unexplained.

The relevant data from one rather extensive experiment on dog 1 are presented in table 1. Urine flow was maintained high to avoid dead space errors. Glomerular filtration rate remained quite constant, indicating a satisfactory physiologic state of the animal.¹ Plasma phosphate concentration was increased stepwise over a range from 1 to 31 mgm. per cent, yet within any group of three periods the rise was gradual. These points are worthy of emphasis as criteria of an adequate experiment and they perhaps explain the divergence of our results and those of previous investigators.

TABLE 1

Experiment on a normal dog which shows the relationship between the quantities of inorganic phosphate and chloride filtered through the glomeruli and the quantities reabsorbed by the tubules and excreted in the urine

Dog 1; 18.7 kgm.; S.A. 0.72 sq.m.

TOTAL CONCURRENT TIME	URINE FLOW	GLOMER- ULAR FILTRA- TION RATE	CHLORIDE				PHOSPHATE-P				PHOSPHATE/ CREATININE CLEARANCE RATIO
			Plasma conc.	Fil- tered	Ex- creted	Reab- sorbed	Plasma Conc.	Fil- tered	Ex- creted	Reab- sorbed	
min.	cc./min.	cc./min.	mgm. %	mgm./ min.	mgm./ min.	mgm./ min.	mgm. %	mgm./ min.	mgm./ min.	mgm./ min.	
75	Infuse: creatinine, 0.83%; glucose, 4.0%; phosphate, 0.0 M.; at 5.0 cc./min.										
90-100	6.8	85.6	364	312	1.5	310	1.25	1.07	0.01	1.06	0.004
100-110	6.8	83.3	366	305	1.5	303	1.16	0.97	0.01	0.96	0.006
110-120	7.0	82.1	367	301	1.7	299	1.02	0.84	0.01	0.83	0.007
121	Infuse: creatinine, 0.83%; glucose, 2.0%; phosphate, 0.05 M.; at 5.0 cc./min.										
135-145	9.2	83.4	371	309	2.5	306	2.75	2.29	0.04	2.25	0.016
145-155	9.7	81.8	373	305	2.4	302	3.70	3.05	0.29	2.76	0.094
155-165	8.7	83.9	374	314	2.5	311	4.64	3.89	0.79	3.10	0.204
166	Infuse: creatinine, 0.55%; phosphate, 0.08 M.; at 7.5 cc./min.										
180-190	6.7	82.1	370	304	2.3	301	9.34	7.66	4.62	3.04	0.602
190-200	7.6	83.1	374	311	2.4	302	11.6	9.64	6.35	3.29	0.660
200-210	8.2	83.6	372	310	3.1	307	13.0	10.9	7.84	3.06	0.722
211	Infuse: creatinine, 0.41%; phosphate, 0.125 M.; at 10.0 cc./min.										
225-235	9.2	78.1	360	281	2.1	279	23.9	18.7	15.7	3.00	0.843
235-245	10.0	78.0	360	281	2.6	278	27.9	21.7	18.4	3.30	0.846
245-255	10.0	75.7	361	273	2.2	271	31.7	24.0	20.8	3.20	0.866

It is evident from the first 3 periods of table 1 that, of the phosphate filtered normally, essentially all was reabsorbed, 1 per cent or less appeared in the urine. Plasma phosphate was extremely low in the initial periods of this experiment, undoubtedly as a result of the infusion of glucose (9). However, in other animals in which plasma phosphate was in a more normal range of 3 to 4 mgm.

¹ The slight drop in glomerular clearance noted in the last 3 periods was accompanied by manifestations of severe tetany. Tetany in our animals was treated by the administration of calcium gluconate intravenously and subcutaneously at the end of the experiment. As a consequence no animals were lost from this cause, although tetany, at times, was extreme.

per cent, similarly low excretion rates have been noted. It should be emphasized that these animals had been fasted some 18 hours before experimentation, a factor of importance in determining the low excretion rate.

As plasma phosphate concentration and hence the amount filtered was increased, the quantities excreted and reabsorbed increased (second group of 3 periods). In the last 6 periods a reabsorptive maximum of approximately 3 mgm. per minute was attained, the excess being excreted in the urine.

The significant features of this experiment are more readily comprehended by plotting the quantity of phosphate reabsorbed by the tubules and excreted in the urine against the quantity filtered through the glomeruli, i.e., the amount presented to the tubules. Such a plot, in figure 1, emphasizes the limited reabsorptive capacity of the renal tubules for phosphate and the quantitative delivery of the excess into the urine.

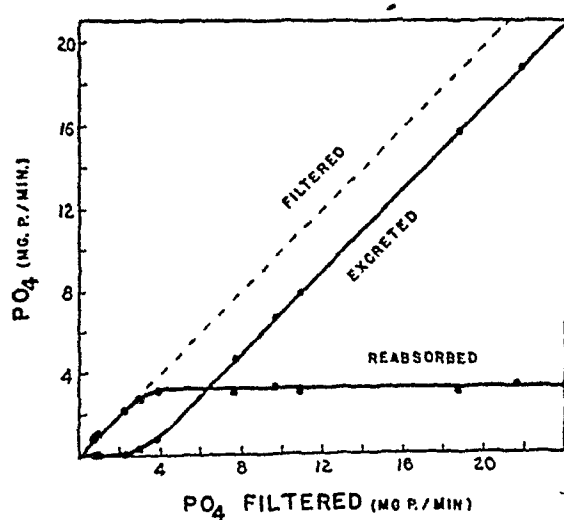


Fig. 1. The renal reabsorption and excretion of inorganic phosphate as a function of the quantity filtered. Dog 1.

A series of similar but shorter experiments, totaling 78 clearance periods, were performed on dogs 4 and 5, the results of which are presented in figures 2A and B. In figure 2A the quantity of phosphate reabsorbed per minute is plotted against plasma phosphate concentration; the range of plasma concentration extending from 2 to 52 mgm. per cent. It is evident that these dogs reabsorbed on an average somewhat more phosphate than the preceding one, a little over 4 mgm. per minute. However, a limitation of reabsorptive capacity is equally evident. The scatter of the data is scarcely more than might be expected from random experimental error. However, in the periods below about 16 mgm. per cent, either glucose or saline were incorporated at random in the infusions to render them approximately isotonic. As will be evident later, some variability may have been introduced by the addition of glucose.

In figure 2B the ratio of the phosphate to the creatinine clearance is plotted against plasma phosphate concentration. The increase in phosphate clearance

is evident as one increases phosphate in the plasma; the asymptote which is apparently approached at infinitely high plasma concentrations is the glomerular clearance, as indicated by the dotted line.

Stability of the phosphate reabsorptive mechanism despite alterations of total electrolyte pattern. The studies just described have been performed by superimposing an elevated plasma phosphate concentration upon an otherwise normal

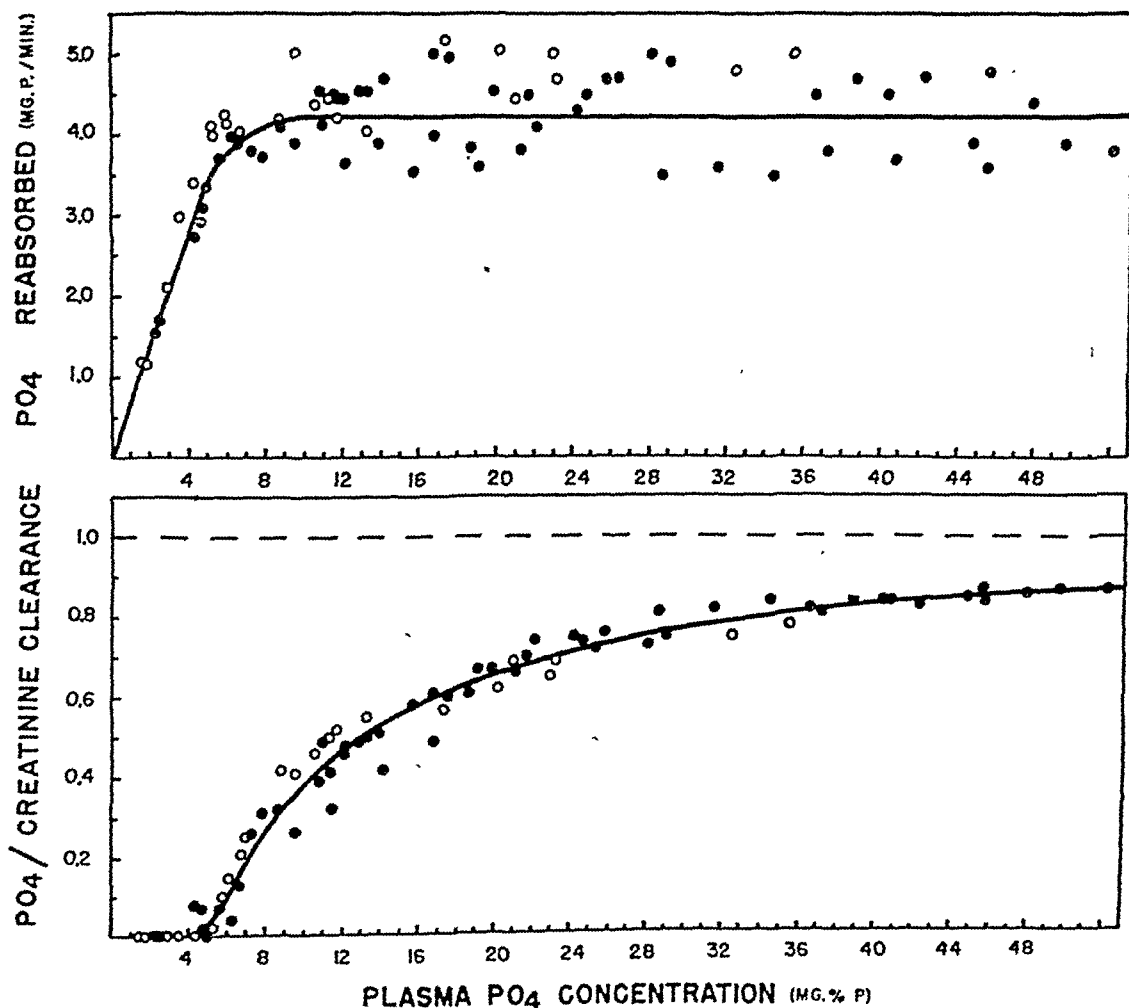


Fig. 2. Above: the renal reabsorption of inorganic phosphate as a function of plasma concentration; dots, experiments on dog 5; circles, experiments on dog 4.

Below: the phosphate/creatinine clearance ratio as a function of plasma concentration. The data for the two figures were derived from 78 clearance comparisons on the two dogs.

electrolyte pattern. It might be presumed that disturbance of this electrolyte pattern would alter the reabsorptive capacity for phosphate. Chloride, as the most abundant anion of plasma, has accordingly been studied during saturation of the reabsorptive mechanism for phosphate.

From table 1 it is apparent that saturation of the phosphate reabsorptive system is without effect on reabsorption or excretion of chloride. The decreased chloride reabsorption, noted in the last 3 periods of this experiment, results from

a combined fall in both plasma concentration and glomerular filtration rate and not from any increased excretion. This experiment has been reversed as indicated in table 2. The phosphate reabsorptive system has been maintained saturated and chloride reabsorption and excretion increased by infusing sodium chloride. Considerable increases in chloride excretion and reabsorption are without effect on the reabsorptive capacity for phosphate. Similar conclusions may be drawn from experiments on bicarbonate (table 4; figs. 3A and B) presented below in another connection. Thus the phosphate reabsorptive mechanism is relatively stable and independent of moderate shifts in general electrolyte pattern.

TABLE 2

Experiment on a normal dog which illustrates the independence of the phosphate and chloride reabsorptive mechanisms

Dog 3; 17.5 kgm.; S.A. 0.76 sq.m.

TOTAL CONCURREN- T TIME	URINE FLOW	GLOMER- ULAR FILTRATION RATE	PHOSPHATE- P				CHLORIDE			
			Plasma conc.	Filtered	Excreted	Reab- sorbed	Plasma conc.	Filtered	Excreted	Reab- sorbed
min.	cc./min.	cc./min.	mgm. %	mgm./ min.	mgm./ min.	mgm./ min.	mgm. %	mgm./ min.	mgm./ min.	mgm./ min.
80	Infuse: creatinine, 0.55%; phosphate, 0.066 M; NaCl, 0.0%; at 7.5 cc./min.									
100-110	11.1	81.5	16.9	13.7	10.2	3.5	373	304	0.4	304
110-120	10.2	82.5	17.2	14.2	10.7	3.5	373	308	0.4	308
120-130	7.4	84.5	17.2	14.5	10.9	3.6	372	313	0.4	313
						3.5				
131	Infuse: creatinine, 0.55%; phosphate, 0.066 M; NaCl, 2.5%; at 7.5 cc./min.									
150-160	10.1	88.2	17.5	15.4	11.7	3.7	399	352	14.6	337
160-170	10.1	91.1	17.4	15.8	12.1	3.7	410	374	20.2	354
170-180	10.6	90.8	17.4	15.8	12.3	3.5	418	380	25.6	354
180-190	11.3	91.2	17.3	15.8	12.4	3.4	425	388	30.5	357
190-200	11.5	91.7	17.3	15.9	12.5	3.4	431	395	31.2	364
						3.5				

Similarities between the phosphate and glucose reabsorptive mechanisms. Because of the similarity of the phosphate and glucose reabsorptive mechanisms and because glucose reabsorption is presumed to be dependent upon a process of tubular phosphorylation (1), we have studied the effects of simultaneous saturation of the glucose mechanism upon the tubular transport of phosphate. In these experiments, presented in table 3, the plasma phosphate concentration has been maintained at a level sufficient to ensure tubular saturation. Glucose has then been infused in increasing amounts to saturate its reabsorptive system. It is evident from table 3, experiment 1, that as glucose reabsorption increased from 83 to 240 mgm. per minute, phosphate reabsorption diminished from 2.35 to 1.41 mgm. per minute. Such a result is compatible with the view that the

TABLE 3

Experiments on a normal dog which illustrates the depression of phosphate reabsorption by simultaneous saturation of the glucose reabsorptive mechanism and the reversal of this depression by phlorizin

Dog 1; 18.7 kgm.; S.A. 0.72 sq. m.

EXPERIMENT NUMBER	TOTAL CONCURRENT TIME	URINE FLOW	GLOMERULAR FILTRATION RATE	PHOSPHATE-P				GLUCOSE			
				Plasma conc.	Filtered	Excreted	Reabsorbed	Plasma conc.	Filtered	Excreted	Reabsorbed
	min.	cc./min.	cc./min.	mgm. %	mgm./min.	mgm./min.	mgm./min.	mgm. %	mgm./min.	mgm./min.	mgm./min.
1	60	Infuse: creatinine, 0.8%; phosphate, 0.07 M.; glucose, 0.0%; at 5.0 cc./min.									
	80-90	10.4	72.8	12.2	8.88	6.66	2.22	115	83.7	1.0	82.7
	90-100	11.4	73.8	12.8	9.52	7.04	2.48	115	84.8	1.1	83.7
							2.35				83.2
	101	Infuse: creatinine, 0.8%; phosphate, 0.07 M.; glucose, 4.0%; at 5.0 cc./min.									
	115-125	7.7	80.0	13.0	10.4	8.23	2.17	162	130	1.3	129
	125-135	5.7	80.6	12.8	10.3	8.26	2.04	170	137	1.4	136
							2.10				132
	136	Infuse: creatinine, 0.8%; phosphate, 0.07 M.; glucose, 10.0%; at 5.0 cc./min.									
	150-160	6.3	85.8	12.8	11.0	9.13	1.87	247	212	7.5	205
	160-170	6.8	84.7	12.5	10.6	9.04	1.56	257	218	11.0	207
							1.72				206
	171	Infuse: creatinine, 0.8%; phosphate, 0.07 M.; glucose, 16.0%; at 5.0 cc./min.									
	185-195	10.5	82.4	12.3	10.1	8.75	1.35	352	290	48.0	242
	195-205	10.4	80.3	12.3	9.87	8.40	1.47	376	302	63.0	239
							1.41				241
2	75	Infuse: creatinine, 0.5%; phosphate, 0.068 M.; glucose, 0.0%; at 7.5 cc./min.									
	95-100	8.9	73.5	19.3	14.2	11.3	2.90	115	85	1.0	84.0
	105-115	7.7	77.0	19.2	14.8	11.8	3.00	119	92	2.0	90.0
							2.95				87.0
	116	Infuse: creatinine, 0.5%; phosphate, 0.068 M.; glucose, 5.0%; at 7.5 cc./min.									
	135-145	7.7	85.3	17.8	15.2	13.4	1.80	225	192	4.0	188
	145-155	7.3	83.1	17.2	15.2	13.4	1.80	257	226	10.0	216
							1.80				202
	156	Infuse: creatinine, 0.5%; phosphate 0.068 M.; glucose, 13.5%; at 7.5 cc./min.									
	175-185	15.1	83.9	15.9	14.1	12.0	2.10	622	553	328	225
	185-195	13.5	85.9	15.8	13.6	11.7	1.90	604	519	306	213
							2.00				219

TABLE 3—Concluded

EXPERIMENT NUMBER	TOTAL CONCURRENT TIME	URINE FLOW	GLOMERULAR FILTRATION RATE	PHOSPHATE-P				GLUCOSE			
				Plasma conc.	Filtered	Excreted	Reabsorbed	Plasma conc.	Filtered	Excreted	Reabsorbed
	min.	cc./min.	cc./min.	mgm. %	mgm./min.	mgm./min.	mgm./min.	mgm. %	mgm./min.	mgm./min.	mgm./min.
2	198	1.0 gram phlorizin intravenously									
	215-225	12.2	67.6	18.0	12.2	8.6	3.60	549	371	370	1
	225-235	10.8	65.6	18.4	12.1	8.8	3.30	540	354	346	8
							3.45				5
3	93	Infuse: creatinine, 0.42% phosphate, 0.05 M.; glucose, 10.0%; at 10.0 cc./min.									
	115-125	18.2	82.3	14.6	12.0	9.85	2.15	531	437	218	219
	125-135	16.7	78.5	14.5	11.4	9.30	2.10	496	389	174	215
	135-145	14.0	78.3	14.3	11.2	9.17	2.03	472	370	147	223
							2.09				219
	148	1.0 gram phlorizin intravenously									
	165-175	10.0	51.5	16.9	8.71	5.63	3.08	425	219	218	1
	175-185	10.9	50.6	17.7	8.96	6.05	2.91	425	215	208	7
	185-195	10.0	47.4	19.1	9.05	6.18	2.87	451	214	208	6
							2.95				5

two reabsorptive mechanisms interact as a result of some competition for an element common to the two mechanisms.

Phlorizin is known to block the reabsorption of glucose without seriously affecting the reabsorption of phosphate. The results obtained on repeating this experiment as before, but terminating it by administering phlorizin, are given in experiment 2. Note that as glucose reabsorption increased from 87 to 219 mgm. per minute, phosphate reabsorption diminished from 2.95 to 2.0 mgm. per minute. As shown by the last two periods essentially complete blocking of glucose reabsorption by phlorizin was attended by increased phosphate reabsorption, an increase, as a matter of fact, to a value higher than the initial control. This suggests that the normal reabsorption of even 87 mgm. of glucose per minute diminished the capacity of the tubules to reabsorb phosphate. This effect of phlorizin, in restoring the reabsorption of phosphate depressed by glucose, is further illustrated by experiment 3. These results indicate that, while there are some factors common to the reabsorptive mechanisms for glucose and phosphate, these two mechanisms cannot be identical throughout, for the former is phlorizin sensitive while the latter is not.

As was mentioned earlier, it is possible that the glucose depression of phosphate reabsorption may have introduced some variability into the experiments plotted in figure 2, for approximately half the points below 16 mgm. per cent plasma phosphorus were obtained in experiments in which glucose had been incorporated in the infusions to render them isotonic. The amount of this added glucose was small, however, and comparison of the data from experiments with

glucose and with chloride indicated that any tendency for the glucose to lower the values for reabsorption in these early experiments was too small to be distinguished from the random experimental error.

Independence of the tubular reabsorptive mechanism for phosphate and the mechanism for acidifying the urine. It is generally recognized that phosphate is the chief buffer present in normal urine and that the elimination of strong mineral acids from the body depends in part on the ability of the kidney to excrete urine containing phosphate in the form of an acid salt (11). Harrison and Harrison (8) maintain that the reabsorptive capacity of the renal tubules for phosphate is diminished in conditions of acidosis and account for the known increased

TABLE 4

Experiment which illustrates the failure of a rapid shift from acidosis to alkalosis to affect the renal reabsorptive capacity for inorganic phosphate

Dog 1; 18.7 kgm. S.A. 0.72 sq. m.

TOTAL CONCURRENT TIME	URINE FLOW	GLOMER- ULAR FILTRATION RATE	URINE pH	PLASMA-CO ₂ COMBINING POWER	PHOSPHATE-P			
					Plasma conc.	Filtered	Excreted	Reab- sorbed
min.	cc./min.	cc./min.		vol. %	mgm. %	mgm./min.	mgm./min.	mgm./min.
80	Infuse: creatinine, 0.55%; phosphate, 0.066 M.; at 7.5 cc./min.							
100-110	12.3	96.7	5.61	45.7	14.6	14.1	10.9	3.2
110-120	13.7	90.0	5.55	45.7	14.2	12.8	9.8	3.0
120-130	13.8	89.5	5.50	45.8	14.2	12.7	9.3	3.4
								3.2
131	Infuse: creatinine, 0.55%; phosphate, 0.066 M.; bicarbonate, 4.0%; at 7.5 cc./min.							
150-160	11.0	85.4	7.23	68.3	15.1	12.9	9.5	3.4
160-170	12.8	85.0	7.53	77.7	15.3	13.0	9.5	3.5
170-180	14.1	80.2	7.63	84.5	15.8	12.7	9.6	3.1
180-190	12.8	79.7	7.73	89.5	15.8	12.6	9.4	3.2
190-200	12.8	74.6	7.78	93.0	15.8	11.8	8.8	3.0
								3.2

phosphate elimination early in acidosis on this basis. Since the point is fundamental to an understanding of the renal contribution to acid-base balance, we have compared the phosphate reabsorptive mechanism under conditions of acidosis and alkalosis. Our experiments on acidosis have been performed on animals to which 500 cc. of 1 per cent hydrochloric acid had been administered by stomach tube each day for 4 or 5 days, including the day of the experiment. For the experiments on alkalosis, similar amounts of 1 per cent sodium bicarbonate were administered.

A preliminary experiment which indicates that the maximal reabsorptive capacity for phosphate is unchanged by an acute shift from acidosis to alkalosis is given in table 4. Phosphate was infused throughout the experiment at such

a rate as to maintain plasma concentrations relatively constant between 14 and 16 mgm. per cent. After three control periods of moderate acidosis, indicated by a CO_2 combining power of 45 vol. per cent, sodium bicarbonate was added to the infusion to produce a progressively rising alkali reserve, reaching a final value of 93 vol. per cent. In the control periods the urines were quite acid, in the later periods they were alkaline. The significant feature is that the quantity of phosphate reabsorbed was the same during the initial periods of acidosis and the final ones of alkalosis, averaging 3.2 mgm. of phosphate phosphorus per minute in each instance.

While the maximal reabsorptive capacity for phosphorus is evidently the same for conditions of acidosis and acutely produced alkalosis, it was felt that some difference in the kinetics of the mechanism might lead to a more gradual satura-

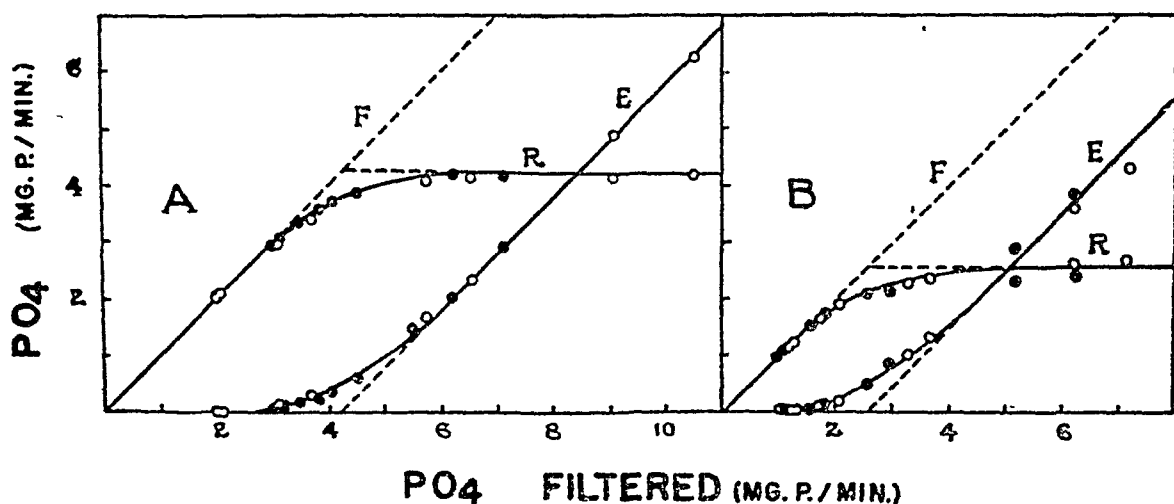


Fig. 3. Comparisons of the renal reabsorption and excretion of inorganic phosphate under conditions of acidosis, circles, and alkalosis, solid dots. *F*, quantity filtered; *E*, quantity excreted; *R*, quantity reabsorbed. Fig. 3A, dog 2; solid dots, plasma CO_2 combining power 61 vol. per cent and urine pH above 7.4; circles, plasma CO_2 combining power 32 vol. per cent and urine pH below 6.3. Fig. 3B, dog 1; solid dots, plasma CO_2 combining power 63 vol. per cent and urine pH above 7.6; circles, plasma CO_2 combining power 27 vol. per cent and urine pH below 5.9.

tion of the system, and hence to a more pronounced rounding of the reabsorptive curve within the physiological range of plasma phosphate concentration. Accordingly, comparisons of the significant part of the reabsorptive curve above and below saturation levels were made in dogs 1 and 2 under conditions of acidosis and alkalosis. These experiments are summarized in graphic form in figures 3A and B.

The two experiments on each of the dogs are distinguished by the solid and open symbols. In figure 3A, the solid dots represent one experiment on dog 2 in which plasma CO_2 combining power was 61 vol. per cent and urine pH varied from 7.4 to 7.9. The hollow dots represent a repetition of this experiment under conditions of acidosis, in which the CO_2 combining power was 32 volumes per cent and urine pH varied from 5.1 to 6.3. In figure 3B is presented a similar

pair of experiments on dog 1 in equivalent states of alkalosis and acidosis.² The identity of the characteristics of the reabsorptive processes in acidosis and alkalosis is sufficiently evident to require no further comment.

These experiments indicate that the increased excretion of phosphate in acidosis results from extra-renal factors. This view is emphasized by the experiment summarized in table 5. In this experiment the inulin clearance was used as a measure of glomerular filtration rate because of the appreciable buffering capacity of creatinine in highly acid urines. The severity of the acidosis is indicated by the arterial pH of 7.30 and a CO₂ combining power of 29 vol. per cent. The titratable acidity was determined by titrating the urines to the pH of the plasma electrometrically; and, expressed in millimols per minute, is a measure of the activity of those renal base conserving mechanisms dependent on urinary buffers.

TABLE 5

Experiment on an acidotic dog which evaluates the rôle of buffer salt and ammonia excretion in the renal conservation of base under conditions of normal and elevated plasma phosphate

Dog 2; 18.7 kgm.; S.A. 0.71 sq. m.

TOTAL CONCURRENT TIME	URINE FLOW	GLOMERULAR FILTRATION RATE	URINE pH	PLASMA pH	PLASMA CO ₂ COMBINING POWER	PHOSPHATE-P				URINE	
						Plasma conc.	Filtered	Excreted	Reabsorbed	Titratable acid	Ammonia
min.	cc./min.	cc./min.			vol. %	mgm. %	mgm./min.	mgm./min.	mgm./min.	mM./min.	mM./min.
115	Infuse: inulin, 0.24%; NaCl, 0.9%; phosphate 0.0 M.; at 7.5 cc./min.										
140-155	4.20	66.4	5.27	7.30	29.0	3.42	2.27	0.01	2.26	0.005	0.053
155-170	2.86	65.5	5.40			3.25	2.13	0.01	2.12	0.002	0.041
171	Infuse: inulin, 0.24%; phosphate 0.10 M.; at 7.5 cc./min.										
195-210	4.87	63.3	6.30			24.1	15.3	11.1	4.20	0.176	0.034
210-225	6.00	65.5	6.32			25.1	16.5	12.4	4.10	0.183	0.033
225-240	6.66	64.5	6.34			26.2	16.9	12.9	4.00	0.198	0.031
240-255	6.46	61.5	6.33			27.9	17.2	13.6	3.60	0.208	0.028

In the two control periods of table 5, despite the severity of the acidosis and the normal plasma phosphate concentration, essentially all the filtered phosphate was reabsorbed, less than 0.5 per cent appearing in the urine. As a consequence of the absence of buffering substance in the urines, although the pH was low (5.27 to 5.40), the titratable acidity was negligible. It should be emphasized that these animals were fasted 18 hours prior to experimentation. In an absorptive state some phosphate would obviously have been available for excretion in the initial two periods. In the last four periods, in which plasma phosphate was elevated to very high levels with the consequent excretion of large amounts of phosphate, the titratable acidities rose to high levels: in the last period, amounting to 0.208 millimol per minute. This amounts to the excretion of 300 cc. of 1.0 normal acid per day. It is evident that the availability of phos-

² Glomerular filtration rates were nearly the same in each of the two series of experiments.

phate and not the severity of the acidosis, is the factor which determines the extent to which this buffer is excreted in correcting an acidosis produced by mineral acid.

Table 5 likewise shows the activity of the renal ammonia mechanism in correcting the acidosis. In the initial periods, ammonia excretion accounts for some 10 times the saving of base effected by buffer excretion. In the succeeding periods, however, both the relative and absolute contributions of the ammonia mechanism are subordinated to the buffer mechanism when phosphate is available in excess.

DISCUSSION. The experiments presented indicate that the renal reabsorptive mechanism for phosphate, like that for glucose (16), is relatively stable and limited in its transport capacity. Similarities between the two systems extend somewhat further than this, for the two substances compete in reabsorption. The explanation of Shannon and Fisher (16) for both limited reabsorptive capacity and competition between reabsorbed materials is based upon an assumed intracellular component present in limited and fixed amount with which these sub-

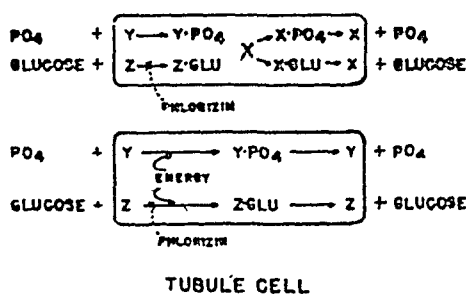


Fig. 4. Possible explanations for competition between phosphate and glucose in renal reabsorption.

stances combine in the process of reabsorption. The rate of reabsorption, when the mechanism is saturated, is presumed to be set by the rate of decomposition of the complex formed with this intracellular component. Competition between glucose and phosphate in renal reabsorption might therefore be due to some link in the reabsorptive processes common to the two substances. If such is true, there must be an initial step in the reabsorptive process different for glucose and phosphate, for reabsorption of the former is blocked by phlorizin, while that of the latter is not.

Accordingly one might postulate some variation of the scheme shown in the upper part of figure 4. The blocking of glucose reabsorption by phlorizin would tend to free this common component *x* for the sole transport of phosphate, explaining the observed restoration of phosphate reabsorption initially depressed by glucose (table 3). Another explanation, suggested by the work of Selkurt (15), indicates that there may be competition on the basis of available energy, as indicated in the lower part of figure 4. If the glucose reabsorptive mechanism is blocked by phlorizin, the energy originally diverted into these channels becomes available for the phosphate system. Glycin and phosphate do not com-

pete in reabsorption, and, while this might be used as an argument against competition on an available cellular energy level; it might only be an indication that these two substances are reabsorbed by different renal cells. Selection between these and other explanations does not seem possible at present.

The slight rounding of the reabsorptive curve as the maximum is approached (see fig. 1) is quite similar to that observed for glucose in man by Smith (18) and perhaps has a similar explanation. Smith maintains that it is due to a disparity between the filtering capacity of individual glomeruli and the reabsorptive capacity of their attached tubules. Thus the reabsorptive capacity of certain nephrons may be exceeded at low plasma concentrations, while that of others requires higher concentrations for saturation. Accordingly, there is a splay in the tubular titration curve. This rounding of the curve is less evident in a mass plot, such as that in figure 2, though evident in the majority of the individual experiments making up that plot. It is quite apparent in figures 3A and B. Whatever may be the cause of the splay in the reabsorptive curve it obviously is the basis of the indefinite excretory threshold. The splay in the curve also emphasizes the necessity for loads safely in excess of reabsorptive capacity, in measuring tubular maximum reabsorptive capacity for phosphate.

The relative stability of the reabsorptive mechanism for phosphate and its independence of the general electrolyte pattern and acid base relations of the plasma have interesting implications. It indicates that phosphate, which normally makes up considerably less than one per cent of the plasma electrolyte, is handled by the kidney in a specific fashion and not merely as an indifferent plasma electrolyte. This is not surprising in the light of the important functions which phosphate subserves within body cells. The conservation of a certain basic store of circulating inorganic phosphate, with provision for the excretion of any excess that may be taken into the body, seems admirably served. Phosphate is no less valuable to the body in conditions of acidosis than alkalosis and its conservation no less important. Its function as a significant urinary buffer, permitting the excretion of acid and the conservation of base, is peculiar to phosphate by virtue of the fact that it is the buffer which normally is present in the body in excess.

Our finding of identical characteristics of the reabsorptive process in acidosis and alkalosis is most reasonable when one considers the work on the amphibian kidney from the Richards school. Thus Walker and Hudson (22) showed in *necturus* that phosphate is reabsorbed in the proximal tubule, while Montgomery and Pierce (10) showed that acidification of the urine takes place in a very localized segment of the distal tubule. Thus the reabsorption of phosphate and the acidification of the urine are not only physiologically distinct processes, as indicated by our results, but are localized in morphologically separate portions of the renal tubule, as indicated by the work on the amphibian kidney.

It is generally conceded that acidosis increases the excretion of phosphate. However, that increase is limited to the first few days of acidosis, phosphate excretion diminishing thereafter accompanied by a rise in ammonia excretion (11). In the absence of a change in the renal reabsorptive process this can mean only 1,

an increase in the urine/fecal excretion ratio and/or 2, the mobilization and loss of a limited store of labile body phosphate.

Harrison and Harrison (7, 8) lay great emphasis on the renal threshold as the prime determinant of plasma phosphate concentration. While in a sense this is true, plasma phosphate is equally determined by the rate of absorption from the gut and the equilibrium between circulating phosphate and intracellular or skeletal phosphate. Thus a change in plasma phosphate concentration is no indication per se of a parallel change in renal threshold. For example, the infusion of glucose lowers plasma phosphate and as a consequence diminishes phosphate excretion (9). Similar effects are produced by hyperventilation (6), while breathing CO₂ raises plasma phosphate and increases excretion (6). These changes are indications of altered equilibria between phosphate stores and circulating phosphate, rather than alterations in the renal mechanism governing phosphate excretion. Presumably the major effect of acidosis on phosphate excretion is of this character.

Any critical analysis of the contrary results of the Harrisons (8) of the effects of acidosis on tubular reabsorption of phosphate is impossible because of their limited presentation of data. Their figures of the quantities of phosphate reabsorbed per unit time under varying conditions are without significance, unless it is known that the load presented to the tubules exceeds the reabsorptive capacity by a safe margin. These facts cannot be derived from their published data.

SUMMARY

1. The renal tubular reabsorption of inorganic phosphate has been assessed at plasma levels from 1 to 52 mgm. of phosphate phosphorus per 100 cc.

2. Phosphate is reabsorbed by an active mechanism which exhibits a limitation of transfer capacity. When the tubular load exceeds the reabsorptive capacity, the excess is excreted quantitatively. The maximal rate of reabsorption is attained at somewhat higher loads than are necessary to initiate excretion. As a consequence no sharp renal threshold exists.

3. Phosphate and glucose compete in renal reabsorption. Yet phlorizin, which blocks the reabsorption of glucose, is without effect or may increase slightly the reabsorption of phosphate. This indicates that whatever may be the basis of the competition, the renal mechanisms cannot be identical in their entirety.

4. Considerable change in the electrolyte and acid base pattern of the plasma are without effect on the kinetics of the reabsorption of phosphate.

REFERENCES

- (1) CORI, C. F. AND G. T. CORI. *Ann. Rev. Biochem.* **10**: 151, 1941.
- (2) FAY, M., V. G. BEHRMANN AND D. M. BUCK. *This Journal* **136**: 716, 1942.
- (3) FISKE, C. H. AND Y. SUBBAROW. *J. Biol. Chem.* **66**: 375, 1925.
- (4) FOLIN, O. *J. Biol. Chem.* **82**: 83, 1929.
- (5) FOLIN, O. AND H. WU. *J. Biol. Chem.* **38**: 81, 1919.
- (6) HALDANE, J. S., V. B. WIGGLESWORTH AND C. E. WOODROW. *Proc. Roy. Soc. B.* **96**: 15, 1924.

- (7) HARRISON, H. E. AND H. C. HARRISON. J. Clin. Investigation 20: 47, 1941.
- (8) HARRISON, H. E. AND H. C. HARRISON. This Journal 134: 781, 1941.
- (9) HARROP, G. A., JR. AND E. M. BENEDICT. J. Biol. Chem. 59: 683, 1924.
- (10) MONTGOMERY, H. AND J. A. PIERCE. This Journal 118: 144, 1937.
- (11) PETERS, J. P. AND D. D. VAN SLYKE. Quantitative clinical chemistry. I. Interpretations. Williams & Wilkins Co., Baltimore, 1931.
- (12) PITTS, R. F. This Journal 106: 1, 1933.
- (13) PITTS, R. F. This Journal 140: 156, 1943.
- (14) SCHALES, O. AND S. S. SCHALES. J. Biol. Chem. 140: 879, 1941.
- (15) SELKURT, E. Personal communication.
- (16) SHANNON, J. A. AND S. FISHER. This Journal 122: 765, 1938.
- (17) SHANNON, J. A., S. FARBER AND L. TROAST. This Journal 133: 752, 1941.
- (18) SMITH, H. W. Lectures on the kidney. University of Kansas, Lawrence, 1943.
- (19) SMITH, P. K., R. W. OLLAYOS AND A. W. WINKLER. J. Clin. Investigation 22: 143, 1943.
- (20) STEINER, A. F., F. URBAN AND E. S. WEST. J. Biol. Chem. 98: 289, 1932.
- (21) SUMMERSON, W. Personal communication.
- (22) WALKER, A. M. AND C. L. HUDSON. This Journal 118: 167, 1937.

SPONTANEOUS ACTIVITY IN RELATION TO DIET IN THE ALBINO RAT

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Basic to theories postulating a relation between diet and muscular activity is the belief that the composition of the diet makes an important difference. Application of such theories may be offset by the efficient homeostasis of the animal body.

Carbohydrate metabolism normally dominates in hard work; fats may play an important rôle and gluconeogenesis from protein can occur. The materials metabolized probably depend largely upon their relative availability at the time.

Pettenkofer and Voit (1), 1866, proved that protein is not the preferred source of energy. A need for extra protein in hard work is not evident.

Fats have been held in low esteem as fuel for muscular work because ketosis may be induced. However, ketosis in itself is not proven to be deleterious and Markees (2) seems to have shown that diets high in unsaturated fats, such as olive oil or in fats such as butter, do not evoke ketosis in the healthy body.

The literature concerning high fat diet and muscular efficiency is extensive but inconclusive. Keyes (3) after a survey of pertinent reports states, "In the absence of better information it is unwise to allow the percentage of calories derived from fat to rise much above 50 per cent."

Fats resist freezing and are of high caloric value; this would enhance their value in winter and in arctic dietaries if muscular efficiency is as high when they provide the calories.

The best criteria for the effects of diet on work are those of actual performance under controlled conditions; these are not easily achieved when using human subjects.

The albino rat is our most standard laboratory animal. The life span of the rat is about 3 years, and that of man about 60 years; hence 1 year of life in the rat is about 20 years in terms of the human life span, and 3 months is about 5 years.

The investigation herein reported concerns the spontaneous activity in 3 groups of 18 rats each over 3 month periods, 2 groups being fed diets high in fat and one high in protein, compared with their litter mates fed a well-balanced ration. The basal rations were so constituted as to allow maximum growth and reproduction throughout the life span of the rat. For exact composition of the diets see figures 1, 2 and 3.

The cages used are as described by Durrant (4). Each consists of a revolving drum one yard in diameter equipped with a rotary ratchet counter, retiring compartment, food container and clean water. Durrant has shown that the pro-

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portion of energy expended in running the cage is so large a proportion of the total that it may serve as a reliable criterion of the latter.

All cages sat on the same table in a basement laboratory throughout the experiments. The results are presented graphically in figures 1, 2 and 3.

For experiment 1 (Oct. 14–Mar. 5) 18 Wistar strain male albino rats ranging in weight from 50 to 70 grams were placed in the cages for a 14-day trial period, all receiving basal ration. This was for the purpose of typing the animals as to spontaneous activity. The 9 most active were thereafter fed basal ration made

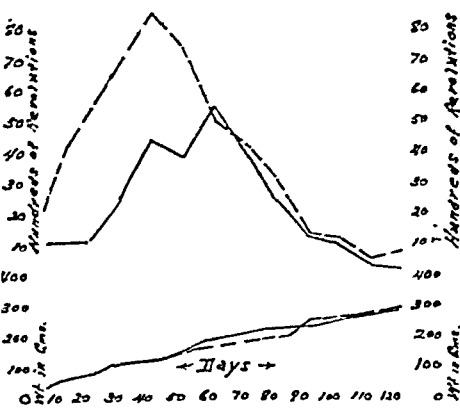


Fig. 1

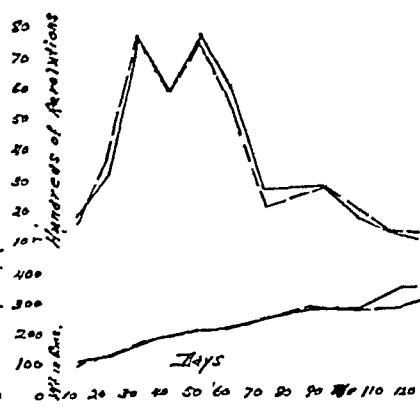


Fig. 2

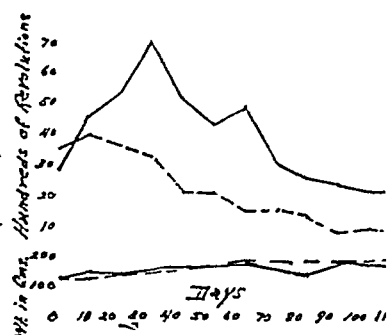


Fig. 3

Fig. 1. Composite activity curves.

Solid line—9 animals fed basal ration. Ground yellow corn 63.75; soy bean oil meal 10.00; linseed oil meal 8.00; wheat middlings 10.00; alfalfa meal 2.00; dried skim milk 5.00; NaCl 0.50; CaCo₃ (fine limestone) 0.50; cod liver oil conc. 0.25; fat content about 4 per cent; fat cal. about 8 per cent; non-fat cal. about 91 per cent.

Interrupted line—9 animals fed basal ration 80 per cent. Lard 20 per cent; fat calories about 41 per cent; non-fat calories about 59 per cent.

Fig. 2. Composite activity curves.

Solid line—9 animals fed basal ration.

Interrupted line—9 animals fed basal ration 66 $\frac{2}{3}$ per cent; lard 33 $\frac{1}{3}$ per cent or about 56 per cent fat calories for first 50 days; and 50 per cent lard or about 72 per cent fat calories for remaining 60 days.

Fig. 3. Composite activity curves.

Solid line—6 animals fed basal ration.

Interrupted line—6 animals fed basal ration to which enough beef muscle was added to make the nutritive ratio: 50 per cent protein calories, 50 per cent non-protein calories.

20 per cent lard by weight. All 18 animals maintained excellent health and vigor throughout the next succeeding 106 days, the lard fed not decreasing in activity to the level of the slower control group (fig. 1).

The body weights in the two groups varied only slightly. At autopsy analysis of serum and liver for fat revealed concentrations about 40 per cent higher in the lard-fed group. There was no difference in the average hemoglobin levels between the 2 groups. Oxalated plasma separated from the blood of lard-fed animals was slightly tinged with red but showed no measurable hemolytic action on red corpuscles of normal rats.

For experiment 2 (Mar. 14–July 10) 18 male albino rats weighing 102 to 150 grams each were placed in the cages for a 10-day activity period on basal ration. The animals were then paired in two groups of 9 each, the pairs having almost identical activity records. One of each pair was changed to basal ration made $33\frac{1}{3}$ per cent lard by weight.

The activity in the two groups ran exactly parallel for the next 50 days after which the lard was increased to 50 per cent. A small reduction in spontaneous activity occurred during the succeeding 60 days (fig. 2). Good health and vigor were maintained by the entire group throughout the 120 days. The weight curves of the two groups varied little. No difference in Hb values was found.

For experiment 3 (Oct. 14–Jan. 30) 18 female rats 150 days of age and 140 to 200 grams in weight were placed in the cages and activity records kept for 20 days after which paired groups of 9 each with nearly equal records were arranged as in experiment 2. Beef muscle to the extent of 50 per cent of the calorific value of the food was added to the diet of one of each pair and the other 9 maintained on control ration.

The rats on high protein showed a decided and persistent decrease in spontaneous activity (fig. 3) from the time they began diet.

All factors other than diet were nearly identical throughout the periods of observation, hence differences in activity probably are due to dietary differences.

SUMMARY

Activity records for 54 rats during about $\frac{1}{2}$ of their life span indicate: 1, that as much as 56 per cent of the calorific value of the food may come from fat and spontaneous activity be maintained at a normal level; 2, that 72 per cent of fat calories depresses activity a little; and 3, that 50 per cent animal protein induces a marked decrease in activity.

REFERENCES

- (1) PETTENKOFER, M. AND C. VOIT. *Ztschr. Biol.* 2: 537, 1886.
- (2) MARKEES, S. *Ztschr. f. Klin. Med.* 135: 516, 1939.
- (3) KEYS, A. *Fed. Proc.* 2: 164, 1943.
- (4) DURRANT, E. P. *This Journal* 70: 344, 1924.

EXPERIMENTAL CHRONIC HYPERTENSION IN THE RABBIT

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Most of the recent studies on experimental hypertension have utilized the dog and rat as objects of study. The rabbit has certain advantages over these two species. The nature of its reproductive cycle makes it particularly suitable for the study of the effect of pregnancy on hypertension. The histological changes originally reported by Goormaghtigh and Grimson (4) to occur in hypertension are particularly striking in this species (3) and worthy of further study. In many laboratories, the rabbit is more readily available than the larger dog. Moreover, the blood pressure may be determined accurately on the rabbit by the use of a blood pressure cuff and stethoscope available to all workers and it is unnecessary to use the more complicated apparatus or arterial punctures used in the rat and dog, respectively. In addition to these advantages, a study of hypertension in different species should throw further light on the general problem of the mechanism of this disorder. In the present paper are described the effects of various manipulations on the kidney on the blood pressure. The implication of the observed results on the general theory of the pathogenesis of hypertension are discussed in the light of these findings.

METHODS. The blood pressure in the adult unanesthetized rabbit may be conveniently and easily determined by the auscultatory method, as described in detail and tested thoroughly by McGregor (9). A wide blood-pressure cuff is applied to the abdomen and the Korotkow sounds as heard through an ordinary stethoscope are used as indicators of the systolic and diastolic pressures. There is no difficulty in obtaining an accurate systolic pressure by this procedure since the first sounds are easily discernible. The determination of the diastolic pressure requires a little more practice. It should be taken at the point where there is a distinct muffling of the sounds. Under these conditions, the values obtained, as shown by McGregor (9), compare well with those observed directly by cannulation of the carotid or femoral arteries. The values for the systolic pressure are about 20 mm. higher than those reported from observations on the smaller vessels of the ear (11) as is to be expected. After a short period of training, the procedure can be carried out without causing struggling or excitement and daily variations are minimal. Determinations on the same animal from day to day agree usually within 5 to 10 mm. Hg.

Hypertension was induced in full-grown rabbits by compression of the renal parenchyma, as described elsewhere (6), by nephrectomy, or by a combination of these procedures. After exposure of the kidney through a dorso-lumbar

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incision, umbilical tape was applied over the poles of the kidney, avoiding the structures at the hilus. Several weeks later, the second kidney was removed or compressed. Hypertension will develop in a large proportion of the animals so treated after an interval of several months.

RESULTS. In table 1 are given the results of observations following various operative procedures designed to throw light on the fundamental nature of chronic renal hypertension in the rabbit.

As noted in the first two experiments, compression or removal of a single kidney both result in elevation of the blood pressure. The extent of this elevation varies in different animals. In some, no demonstrable permanent rise results. In others, moderately severe hypertension ensues. It requires about 10 weeks for the permanent and constant elevation in blood pressure to mani-

TABLE 1

The effect of various experimental procedures on the systolic and diastolic pressures of adult rabbits.

All the results are expressed in terms of millimeters of mercury. The post-operative values are averages obtained between the tenth and fourteenth weeks after the experimental manipulation recorded in the second column.

NUMBER OF ANIMALS	OPERATIVE PROCEDURE	AVERAGE BLOOD PRESSURE	
		Before operation indicated in 2nd column	After operation
		<i>mm. Hg</i>	<i>mm. Hg</i>
12	Constriction of right kidney	115/88	157/119
12	Right nephrectomy	108/82	150/116
6	Left nephrectomy of animal in which right kidney had been constricted previously	144/114	174/137
6	Constriction of left kidney of animal in which right kidney had been removed previously	140/110	168/130
5	Removal of a previously constricted right kidney; left kidney intact	151/120	147/118

fest itself and for this reason the values shown in the table represent averages of at least 6 readings taken during the tenth to fourteenth weeks following the operation. It will be noted that nephrectomy results in a rise in blood pressure comparable to that observed following compression of the kidney. This result speaks against the theory that renal hypertension in the rabbit is a result of the liberation of a pressor substance by the ischemic or injured kidney.

The third and fourth series of experiments cited in table 1 show the increase in blood pressure which follows removal or compression of the remaining kidney after having performed one of these operations on the other kidney. It should be noted that the ultimate effect is the same whether the first operation consists of compression or nephrectomy. The results speak against the currently held view that injury of one kidney results in changes in the other which in turn is responsible for the ultimate development of hypertension.

The final series of experiments cited in table 1 show that the removal of a compressed kidney does not abolish the hypertension induced by this procedure. This experiment demonstrates that compression and nephrectomy are equally effective in raising the blood pressure and that the formation of a pressor substance cannot be responsible in the rabbit for the observed chronic hypertension since removal of the injured kidney which is the assumed source of the hypothetical pressor substance fails to abolish the hypertension.

It should be noted that the compression of the kidney performed in the above experiments was relatively severe. It was of sufficient intensity to deform the normal ellipsoidal shape of the organ. Under these conditions only is the renal dysfunction induced by this procedure comparable to that which follows nephrectomy. If the compression is only moderate, no or only slight elevations in blood pressure will be induced.

Effect of bilateral nephrectomy. To further establish the fact that hypertension is not the result of a circulating pressor substance liberated by the injured kidney or of the failure of the kidney to destroy some circulating pressor substance, the blood pressure in the bilaterally nephrectomized rabbit was determined. The remaining compressed kidney was removed from animals previously nephrectomized unilaterally. In many animals the pressure begins to drop within a few hours and continues to do so until death. However, if traumatic shock be reduced to a minimum this is not always the case as is shown in the following typical experiment which has been observed in about one-third of all the experiments:

A male adult rabbit had a preoperative blood pressure of 120/100. Six months following a right nephrectomy, its blood pressure established itself at a constant level of 145/110 to 150/120. Its left kidney was then compressed. Three months later the blood pressure was 170/140 and it varied from 165/130 to 180/150 during the following month. The remaining compressed kidney was removed on April 5, 1944, at 2 p.m. The subsequent blood pressure readings were as follows:

April 6.....	10 a.m.	200/170
April 6	5 p.m.	180/150
April 7.....	9 a.m.	150/120
April 7.....	5 a.m.	130/100
April 8.....	9 a.m.	100/70

Death occurred several hours after the last recorded reading. This experiment shows that the blood pressure for at least 27 hours can remain at its pre-operative hypertensive level in the absence of all renal tissue.

Results similar to the protocol just quoted were obtained in 5 animals out of a total of 18 similarly treated. In 10 of the remaining animals adhesions of the kidney rendered its ablation difficult and the inevitable trauma and hemorrhage resulted in a survival of the animal for only 12 to 24 hours with a gradual decline in blood pressure, which began immediately after the operation. In the remaining 3 animals, the hypertensive blood level existing pre-operatively was maintained for only a few hours post-operatively after which it gradually declined until the death of the animal 24 to 48 hours later.

DISCUSSION. The results of the present study confirm for the rabbit the results reported previously and the conclusions drawn from a study on the rat (7). They are interpreted to mean that in the rabbit, as in the rat, chronic hypertension is a result of a deficiency of renal tissue rather than of the production by injured or ischemic renal tissue of a pressor substance. This does not preclude the formation in the acute experiment of pressor substances of the nature of renin, hypertension or angiotonin. The presence of these substances in the blood of the renal vein would account for the elevation in the blood pressure seen in acute experiments. However, the data of the present paper are incompatible with the view that the circulation of such pressor substances is responsible for the chronic elevation of the blood pressure in the experimental animal which, after all, is the analogy of the condition seen in human disease.

We have previously suggested (5, 8) that chronic experimental hypertension could best be explained on the assumption that there is a deficiency of some product normally produced by the kidney. This simple view is compatible with all our available experimental data and is further substantiated by the results of the present study. This view has been criticized recently by Braun-Menendez, Fasciolo, Leloir, Munoz and Taquini (1). These authors point out that unilateral nephrectomy is less effective in raising the blood pressure than a unilateral lesion of the kidney. However, as shown above, these procedures are equally effective in the rabbit. The average effect of unilateral nephrectomy on the blood pressure equalled both in degree and duration that which followed a unilaterally inflicted lesion. The above-mentioned authors also suggest that the maintenance of an elevated blood pressure in the absence of both kidneys may be due to the interference of the normal destruction of a pressor substance by the coexisting uremia and that the observed elevation in blood pressure may be a reflex phenomenon. Their most effective argument is the reported reactions in the dog. However, the published experiments carried out on this species are of relatively short duration and merit further investigation.

It should be emphasized that the conclusions reached in the present paper are based on observations made at least 10 weeks following any operative procedure. The immediate effects seen within a few days or weeks following such operations (2, 10, 11) are different from those recorded here, and involve other mechanisms including probably the production of pressor substances by injured or ischemic renal tissue.

SUMMARY

The blood pressure of adult rabbits was followed for three months or more following various operative procedures on the kidneys. The average rise in blood pressure following unilateral compression of the kidney did not exceed that which followed unilateral nephrectomy. Removal of the compressed kidney in a hypertensive animal did not result in lowering of the blood pressure. Rabbits may exhibit hypertension even in the absence of all renal tissue.

The implications of the observed results on our concept of the mechanism of experimental renal hypertension are discussed. In the rabbit, as in the rat,

the available data support the view that chronic hypertension results from a deficiency induced by injury or removal of normal renal tissue and not from the formation of a renal pressor substance. The latter, however, may play a part in causing hypertension in the acute experiment.

REFERENCES

- (1) BRAUN-MENENDEZ, E., J. C. FASCILOLO, L. F. LELOIR, J. M. MUNOZ AND A. C. TAQUINI. Hypertension Arterial Nefrogena. Libreria y Editorial El Ateneo, Buenos Aires, 1943, p. 223.
- (2) DRURY, O. R. J. Exper. Med. 68: 695, 1938.
- (3) DUNIHUE, F. W. AND B. H. CONDON. Arch. Path. 29: 277, 1940.
- (4) GOORMAGHTIGH, N. AND K. S. GRIMSON. Proc. Soc. Exper. Biol. and Med. 42: 227, 1937.
- (5) GROLLMAN, A. Essentials of endocrinology. J. B. Lippincott Co., Philadelphia, 1941, p. 460.
- (6) GROLLMAN, A. Proc. Soc. Exper. Biol. and Med. 57: 102, 1944.
- (7) GROLLMAN, A., T. R. HARRISON AND J. R. WILLIAMS. This Journal 139: 293, 1943.
- (8) GROLLMAN, A. AND C. RULE. This Journal 138: 587, 1943.
- (9) MCGREGOR, L. Arch. Path. 5: 630, 1938.
- (10) MAEGRAITH, G. G. AND F. J. MACLEAN. Brit. J. Exper. Path. 23: 239, 1942.
- (11) PICKERING, G. W. AND M. PRINZMETAL. Clin. Sci. 3: 357, 1938.

FILTRATION ACROSS THE VASCULAR WALL AS A FUNCTION OF SEVERAL PHYSICAL FACTORS¹

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This paper deals with certain permeability characteristics of the blood-tissue fluid barrier in a perfused hind-limb preparation of the rat. The method was developed to obtain direct, continuous determinations of the rate of edema formation in the preparation. By this means were investigated the laws relating variations in the perfusion pressure and the osmotic pressure of the perfusion fluid to the rate of filtration across the barrier.

Several methods have been used to measure the rate of filtration from the vascular system. Many of these have a common feature, viz., the determination of the rate of accumulation of fluid in the extra-vascular tissue. Drury and Jones (1) introduced the use of a plethysmograph to measure directly the increase in tissue fluid volume in some portion of the limb of man. Krogh, Landis and Turner (2) and Landis and Gibbon (3) increased the accuracy of the plethysmographic method by a modification whereby the volume of tissue minus the blood could be determined. Drinker (4) developed a semi-quantitative method for determining the rate at which edema fluid accumulates in the perfused leg of a frog. Ellinger and Heymann (5), and more recently Danielli (6) and Sendery (7), determined the rate of edema formation in a Lawen-Trendelenberg preparation of the frog by measuring the weight at given intervals during a perfusion.

The method described in this paper makes use of the especially prepared hind-limbs of a rat, a perfusion system in which the pressure may be set at any desired level and maintained constant, and a means for recording continuously the weight of the preparation. A description of the technique as used for the frog is given in a previous paper (8).

METHODS. *A. The fluids used.* The colloids used in the experiments have all been considered for use as blood substitutes (cf. 9) and were obtained from the manufacturer in grades suitable for pharmaceutical use.

All the perfusion fluids were prepared by dissolving the colloid in an isotonic, crystalloid solution (either 0.95 per cent NaCl or mammalian Ringer's solution) buffered with sodium bicarbonate and phosphate to pH 7.2 ± 0.2 . In some cases, traces of the dye, T-1824, were added to the perfusion medium.

Colloid osmotic pressure measurements of the solutions were made with the osmometer described by Simms, Zwemer and Lowenstein (10). The membrane employed was DuPont no. 600 cellophane.

B. The preparation. Rats (125 to 175 grams) were anesthetised by intramuscular injections of about 0.1 cc. sodium pentobarbital (5 mgm./cc.).

¹ The work described in this paper was supported in part by the Josiah Macy Jr. Foundation and by the California Fruit Growers' Association.

A mid-ventral incision was made from the sternum to the pelvis; two lateral incisions permitted the displacement of the abdominal viscera and the exposure of the major abdominal blood vessels. To facilitate cannulation of the aorta, the portion of the peritoneum overlying these vessels was cut away.

Two ligatures were passed beneath the aorta and the adjacent post cava, one above the bifurcation of the aorta into the iliacs, and the second below the renal branches. The upper ligature was tied to occlude both aorta and the vena cava. The wall of the aorta between the two ligatures was then snipped with iridectomy scissors, and a glass cannula (ca. 1.5 cm. long and 0.1 cm. in outside diameter) inserted and passed beyond the lower ligature. This ligature was then tied to make a fluid-tight joint. The vena cava was now opened below the second ligature to permit unobstructed outflow. The perfusion was then started. The interval between tying the first ligature and the start of the perfusion was in all cases less than five minutes.

After the perfusion through the limbs was begun, the viscera and the anterior portions were cut away. The completed preparation thus consisted of the musculature, skeleton and skin of the hind-limbs and tail. In several cases the dye T-1824 was added to the perfusion fluid. The skin and muscles of the legs and tail became uniformly tinted with the blue dye, indicating a thorough perfusion.

C. *The weighing and perfusion systems.* The apparatus for perfusion and the method for determining the weight of the preparation during perfusion were essentially the same as previously described (8).

The fluid to be perfused was forced out of a storage reservoir by a compressed mixture of 5 per cent carbon dioxide in 95 per cent oxygen. The perfusion pressure was maintained at a constant level by a mercury blow-off. The fluid on its way to the cannula passed through a dropping chamber to which a manometer was attached. The perfusate passed to the cannula through a six-inch length of rubber latex tubing (1 mm. O. D.) which afforded a flexible connection to assure minimum interference with the weight record.

The weight of the preparation was recorded by a simple spring balance, the vertical displacements of which were traced directly on a slow-moving kymograph drum.

D. *Analysis of the records.* The slope of the line traced on the kymograph drum is directly proportional to the rate of change in weight of the preparation, which in turn is a direct measure of the rate at which fluid is accumulating in the extra-vascular tissues.

The relation between the slope of the line and the absolute rate of increase in weight in grams per minute can be rapidly determined by the following method: the spring had been previously calibrated with known weights, so that the vertical displacement per unit weight can be determined from the record. The horizontal displacement per unit time is determined by a timer on the drum. The weight which is represented by a 2 cm. vertical displacement is divided by the time required for the drum to move 2 cm. This quotient is the rate of change in weight for a line on the kymograph drum having

a slope of 1, and is determined solely by the sensitivity of the spring and the rate of movement of the drum. This quotient is the *rate constant*. To determine the rate of increase in weight represented by any section of the experimental line, it is sufficient to measure the angle between the line and the horizontal, find the tangent of the angle measured (the tangent being the slope) and multiply it by the *rate constant*.

Because of the continuity of the weight record, any change in the rate of accumulation of fluid is apparent on observation and, accordingly, is amenable to quantitative expression by the method outlined above.

EXPERIMENTAL. The descriptions of the experiments are divided into two sections. In section A those experiments giving data relative to the filtration rate as a function of osmotic pressure are described; in section B are included the experiments giving data relative to filtration rate, as a function of perfusion pressure. The following symbols are used to represent the quantitative factors involved: dV/dt , sometimes represented by y , for the filtration rate; h_o for the osmotic pressure; P_p for the perfusion pressure; and E for the rate of change of filtration rate with change in driving force.

In each section, A and B, one experiment is given in detail.

Section A. Filtration rate as a function of osmotic pressure. $y = f(h_o)$. In these experiments the perfusion pressure was kept constant throughout, while each preparation was perfused with solutions of several different dilutions of the same colloid. Thus the rate of edema formation in a single preparation was determined for several concentrations of a colloid.

Experiment 1: *With pectin in saline this relationship was found to be non-linear.* Figure 1, curve A.

The perfusion was begun with a solution containing 0.15 per cent pectin² in 0.9 per cent sodium chloride, buffered to pH 7.2 (soln. 1). The perfusion pressure (P_p) was ca. 87 mm. Hg. Ten minutes later all the blood was washed out and the weight record begun.

Forty-five minutes later, by turning a valve, a second reservoir containing a 0.30 per cent solution of pectin (soln. 2) was connected to the dropping chamber. As indicator for the progress of the more concentrated solution, a small amount of the dye, T-1824, was introduced into the system between the reservoir and the dropping chamber.

The first drop to show color fell from the chamber five minutes later, indicating that by that time all of the fluid in the preparation had been replaced by solution 2. Meanwhile the reservoir containing solution 1 was replaced by one containing a 0.60 per cent solution of pectin (soln. 3) which was connected fifty minutes later to the dropping chamber. An injection of dye was made as in the first case, and in five minutes the fluid leaving the preparation was seen to be blue in color. After forty-five minutes the undiluted 1.5 per cent pectin (soln. 4) was connected to the animal. One hour and fifteen minutes later a colloid-free NaCl

² The pectin solutions were made by saline dilutions of Cutter's 5774-A Pectin. This material is supplied in a 1.5 per cent solution in 0.9 per cent NaCl. I wish to thank Dr. R. B. Clark of the Cutter Laboratories for supplying this material.

solution (soln. 5) was substituted for the last solution. After a total of five hours and ten minutes the experiment was terminated. The preparation was removed from the chamber, disconnected from the cannula and weighed on a simple trip balance. This final weight was found to be 58 grams.

The total increase in weight was determined from the kymograph record of this experiment by measuring the vertical distance between the end and the beginning of the curve. The initial weight of the preparation was determined by subtracting the increase from the final weight.

The angle made by each segment of the kymograph record with the horizontal was measured. These values are listed together with the tangents in table 1. A negative value indicates decrease in weight. To determine the rate of change in weight in grams per minute, the tangent of the angle was multiplied by the *rate constant*. These filtration rates are listed in the fourth column of table 1

TABLE 1
Filtration rate as a function of osmotic pressure—Cutter's Pectin 5774A

ANIMAL	CONC.	ANGLE	TANGENT	y	y	h_o
	gr. %			gm./min.	gm. %/min.	cm. H ₂ O
1	0.15	23°15'	0.430	0.714	0.166	5.0*
	0.30	10°55'	0.193	0.318	0.075	11.4*
	0.60	4°05'	0.071	0.114	0.028	20.0
	1.50	-3°50'	0.067	-0.108	-0.025	50.0*
2	0.25			0.570	0.095	8.5*
	0.50			0.180	0.030	16.5
	0.75			0.108	0.018	25.0
	1.50			-0.060	-0.010	50.0*

* These pressures were determined directly. All others were obtained by interpolation. Each group of data was obtained on a single animal preparation. Thus, the Pectin data represent two animals, while the gelatin data were taken from three.

headed y , grams/min. In order to make the results obtained in this experiment comparable with those obtained in other experiments, the filtration rate per 100 grams of tissue was determined by dividing the values of y , grams/min. by the initial weight of the preparation and multiplying by 100. These values are listed in the column headed y , grams per cent/min.

In a second preparation the filtration rates were determined with perfusion fluids respectively containing 0.25, 0.50, 0.75 and 1.50 per cent pectin. These data are included in table 1.

In the sixth column of table 1 (headed h_o) are listed the osmotic pressures of the solutions used in both preparations.

The values of y , grams per cent/min. for these several concentrations of pectin are plotted against the corresponding values of the osmotic pressure in figure 1. From this curve (A), it may be seen that a solution of pectin having an osmotic pressure of about 20.5 mm. Hg (28 cm. H₂O) would prevent edema formation in the preparations used.

Experiment 2: *With gelatin in saline a similar non-linear relationship was found.* Figure 1, curve B.

Three preparations were used. The values for the filtration rates for the several concentrations of gelatin³ are given in the columns headed y , grams/min. and y , grams per cent/min. in table 2. In the column headed h_o are listed the osmotic pressures corresponding to the several concentrations of gelatin used.

Figure 1, B, represents the value of y , grams per cent/min. for the several values of the osmotic pressure of the gelatin tested. The intercept of this curve shows that a solution of gelatin having an osmotic pressure of about 70 cm. H₂O is required to prevent edema in the preparations.

Experiments 3 and 4: *With bovine albumin in Ringer's solution and with reconstituted human plasma a linear relationship was found.* Figure 2 A and B.

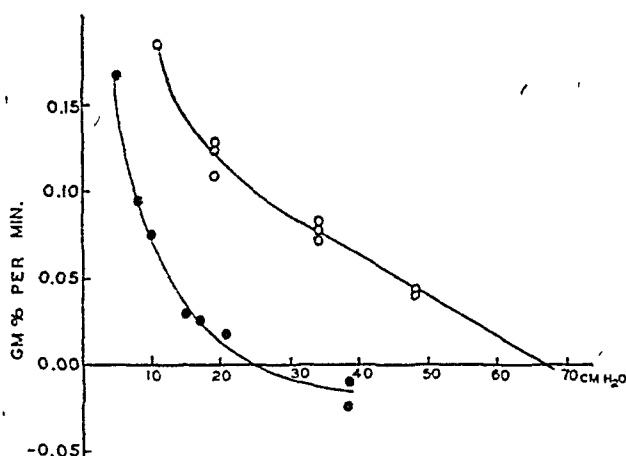


Fig. 1

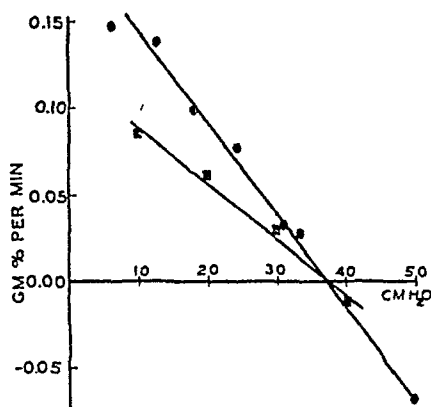


Fig. 2

Fig. 1. Filtration rate as a function of osmotic pressure. Ordinates, y , gram per cent/min.; abscissa, osmotic pressure in centimeters H₂O. Solid ovals, curve A, data for pectin. Open circles, curve B, data for gelatin.

Fig. 2. Filtration rate as a function of osmotic pressure. Ordinates, y , gram per cent/min.; abscissa, osmotic pressure in centimeters H₂O. Ovals, curve A, data for albumin. Squares, curve B, data for plasma.

The data obtained in these experiments are given in table 3, parts A and B, and plotted in figure 2. The albumin⁴ data were obtained from three animals, while the plasma⁵ results were obtained from a single animal.

³ The gelatin solutions used were made by dilution of Knox's P5-2L, packaged in a 6 per cent solution, in isotonic saline. This material was generously supplied by Mr. D. Tournellotte of the Knox Gelatin Company, Johnstown, N. Y.

⁴ The bovine albumin solutions were made by dissolving the dry material in mammalian Ringer's solution and buffering to pH 7.2. The albumin was prepared at the Armour Laboratories by methods developed in the Department of Physical Chemistry, Harvard Medical School, both under contract with the Committee on Medical Research of The Office of Scientific Research and Development.

⁵ The human plasma was made by reconstituting a lyophilized preparation of this material. The several dilutions were made by adding saline to the reconstituted plasma. The samples used were considered to be substandard for human use, and therefore made available for experimental use.

From the figure it is clear that the relationship between y and h_o for both these substances is a linear one. Both lines intercept the osmotic pressure axis at about 32 cm. H_2O .

TABLE 2

~ Filtration rate as a function of osmotic pressure—Knox Gelatin P5-20

ANIMAL	CONC.	y	y	h_o
	gr. %	gm./min.	gm.%/min.	cm. H_2O
1	1.0	0.792	0.108	18.8*
	2.0	0.570	0.077	34.4
	3.0	0.292	0.040	50.0*
2	1.0	0.762	0.127	18.8*
	2.0	0.443	0.074	34.4
	3.0	0.271	0.045	50.0*
3	0.5	0.924	0.185	9.4
	1.0	0.624	0.125	18.8*
	2.0	0.414	0.083	34.4

TABLE 3

Filtration rate as a function of osmotic pressure

ANIMAL	CONC.	y	y	h_o
A. Bovine albumin				
	gm. %	gm./min.	gm. %/min.	cm. H_2O
1	2.0	0.097	0.138	12.6*
	5.5	0.019	0.026	34.4
2	1.0	0.712	0.147	7.0*
	2.0	0.513	0.106	12.6*
	5.0	0.156	0.032	30.5*
3	3.0	0.417	0.089	18.8
	4.0	0.352	0.075	25.0
	8.0	-0.352	-0.075	50.0*
B. Human plasma				
1	0.25C	0.350	0.083	10
	0.50C	0.261	0.062	20*
	0.75C	0.105	0.025	30
	1.00C	-0.067	-0.016	40*

Summary of section A. The results of these first four experiments indicate an inverse relationship between the colloid osmotic pressure of the perfusion fluid and the rate of edema formation. With pectin and gelatin the relationships are non-linear; with bovine albumin and dried human plasma they are linear.

Section B. Filtration rate as a function of the perfusion pressure. $y = f(P_p)$. In these experiments the solution was kept constant through the course of each perfusion while the perfusion pressure was varied.

Experiment 5: *With a colloid-free mammalian Ringer's solution the relationship is linear throughout a pressure range of 60 to 120 cm. H₂O (48 to 88 mm. Hg).* Figure 3.

The perfusion with mammalian Ringer's buffered to pH 7.2 was begun at a pressure of 85 mm. Hg and gradually decreased to 25 mm. Hg by lowering the leveling bulb on the mercury blow-off. The preparation was then placed in the

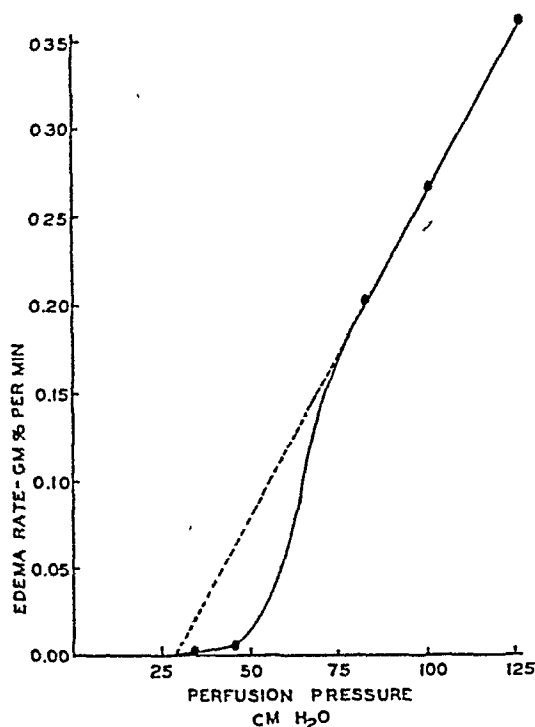


Fig. 3

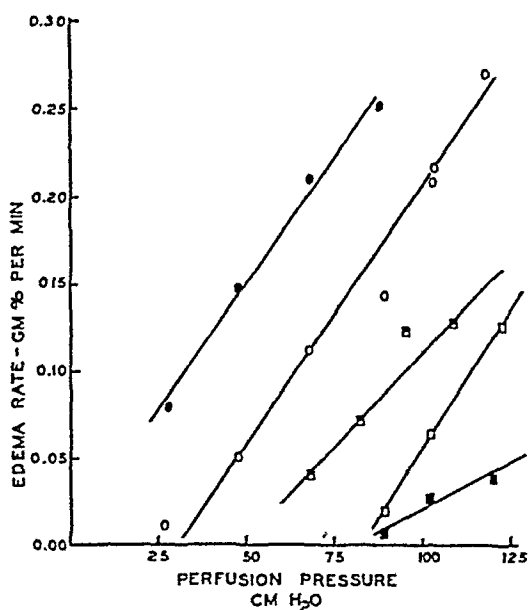


Fig. 4

Fig. 3. Filtration rate as a function of perfusion pressure: data for saline perfusions.

Fig. 4. Filtration rate as a function of perfusion pressure. Solid circles (line A) 2 per cent albumin. Open circles (line B) 3 per cent albumin. Solid squares (line C) undiluted plasma. Half-solid squares (line D) $\frac{1}{2}$ diluted plasma. Open squares (line E) $\frac{1}{2}$ diluted plasma.

chamber and the kymograph record begun. The perfusion was continued at 25 mm. Hg for thirty-five minutes. The pressure was then increased to 34 mm. Hg and the perfusion continued for thirty minutes. The pressure was then raised to 61, 75 and 93 mm. Hg respectively at thirty minute intervals.

The data derived from this experiment are given in table 4. In the first two columns are listed the perfusion pressures in millimeters of mercury and centimeters of water; in the third column, headed y , grams/min., the uncorrected filtration rates; and in the fourth column, headed y , grams per cent/min., the rates of filtration per 100 grams of tissue.

In figure 3 are plotted the values of y , grams per cent/min. as a function of the perfusion pressure, P_p . The three uppermost points fall on a straight line, while

the two points taken at the low pressures do not. The point of interception of the straight line with the pressure axis represents a constant of the system which is probably a function of the loss in pressure between the manometer and the mean pressure in the filtration area.

Experiments 6 and 7: *With 2 and 3 per cent bovine albumin in mammalian Ringer's solution the relationship is linear.* Figure 4, A and B respectively.

In table 5, sections A-1 and A-2 respectively, are listed the data obtained in these experiments. The values of y , grams per cent/min. for the several values of P_p are plotted as lines A and B in figure 4.

These data are satisfied by two parallel straight lines.

Experiments 8, 9 and 10: *With plasma and diluted plasma the relationship is linear.* Figure 4, C, D and E.

In table 5, sections B-1, B-2 and B-3, data are given for experiments with plasma, $\frac{1}{4}$ diluted plasma, and $\frac{1}{2}$ diluted plasma respectively.

These data are plotted as C, D and E of figure 4. They are satisfied by straight lines, the slopes of which are related to the dilution of the plasma.

TABLE 4
Filtration rate as a function of perfusion pressure—Saline

PRESSURE		y	y
<i>mm. Hg</i>	<i>cm. H₂O</i>	<i>gm./min.</i>	<i>gm. %/min.</i>
25	34.0	0.0021	0.004
34	46.3	0.0140	0.031
61	82.8	0.0915	0.202
74	100.5	0.1210	0.268
93	126.0	0.1630	0.363

Summary of section B. The relationship between perfusion pressure and filtration rate is direct and linear. The change in filtration rate for unit change in perfusion pressure is independent of concentration for albumin, but becomes less with addition of greater concentrations of plasma.

DISCUSSION. The filtration rate is a function of the driving force and the filtration area. Under the conditions of the experiments, the filtration area was not used as a dependent variable. It was assumed that no major changes in area resulted from the changes in filtration force imposed on the system because no significant change in rate of flow through the preparation could be detected in most of the experiments. In the case of perfusions with plasma, where major changes in rate of flow do occur, the possibility of change in filtration area is noted, and its contribution to the change in filtration rate is discussed below. For the most part, however, the filtration area is taken to be constant, so that the filtration rate becomes a function of the driving force alone.

This driving force has been defined by the equation of Bellis (11), which may be re-written:

$$F = P_c - h_o - P_t + h_t \quad (1)$$

in which F is the net driving force; P_c and P_t the capillary and tissue hydrostatic pressures respectively; h_o and h_t the osmotic pressures of the perfusion medium and the tissue fluids respectively. It is therefore the resultant of four independent components.

TABLE 5
Filtration rate as a function of perfusion pressure

ANIMAL	PRESSURE		y	y
Section A-1. Bovine albumin 2%. 4/3/44				
	<i>mm. Hg</i>	<i>cm. H₂O</i>	<i>gm./min.</i>	<i>gm. %/min.</i>
1	35	48.7	0.071	0.146
	50	68.0	0.084	0.209
	65	88.8	0.100	0.268
Section A-2. Bovine albumin 3%. 4/3/44				
1	20	27.2	0.005	0.011
	35	48.7	0.023	0.050
	50	68.0	0.052	0.112
	75	103.0	0.100	0.215
2	65	88.8	0.0613	0.142
	75	102.0	0.0915	0.212
	85	116.0	0.1159	0.268
Section B-1. Human plasma, C				
1	65	88.8	0.0033	0.007
	75	102.0	0.0129	0.027
	85	116.0	0.0174	0.037
Section B-2. Human plasma 0.75 × C				
1	50	68.0	0.0269	0.040
	60	81.5	0.0485	0.071
	70	95.0	0.0835	0.123
	80	108.5	0.0855	0.126
Section B-3. Human plasma 0.50 × C				
1	65	88.8	0.0089	0.019
	75	103.0	0.0302	0.064
	90	123.0	0.0582	0.124

In the experiments described in this paper, the two minor components of the driving force, i.e., the tissue pressure and the osmotic pressure of the tissue fluid, were not measured. In all of the perfusions, even those of long duration, in which the total weight of the preparation had more than doubled, there was no decrease in the filtration rate with time. This result is apparently at variance

with those of Drury and Jones (1) and of Landis and Gibbon (3), both of whom report a decrease in the rate of outward filtration of fluid as the tissue becomes more edematous. This difference may be attributed to the distribution of the edema fluid in such a way that the tissue pressure (P_t) in the preparations used does not increase for the amounts of edema normally encountered in the course of an experiment. The osmotic pressure of the tissue fluid (h_t) could change in either one of two ways. Neither of these changes was found in the experiments reported in this paper. The accumulation of filtrate of the perfusion medium in the tissues should lower the concentration of tissue fluid colloids by increasing the volume of the tissue fluid. On the other hand, in the case of certain colloids, there may be an escape of colloidal substance into the tissue fluid, at a rate sufficient to increase the concentration or, at least, the apparent osmotic pressure of the tissue fluid.

The osmotic pressure of the perfusion fluid (h_o) was determined directly, and could be varied by varying the concentrations of colloid in the solutions. The pressure in the filtration area (P_c) was measured indirectly, according to a relationship proposed by Gomez (12), as some function of the perfusion pressure (P_p). In any event, the perfusion pressure could be varied or held constant at will. The filtration rate may therefore be considered as a function of the osmotic pressure and the perfusion pressure. This could be expressed by an equation of the form:

$$dV/dt = f(aP_p - h_o - C) \quad (2)$$

where a is the constant relating the capillary pressure, P_c , to the perfusion pressure, P_p , and C is a constant that includes the osmotic and hydrostatic pressure of the tissue fluid.

In the experiments of the first section, the only variable was the osmotic pressure. From the results obtained with albumin and plasma the nature of the relationship between this variable and the filtration rate per unit tissue may be established.

The use of y , grams per cent/min. makes it possible to compare data obtained from two different animals. The use of such a unit assumes equal filtration area per gram tissue. The validity of this assumption may be judged from the variability of the filtration rate measured during perfusion with saline alone at a pressure of 85 mm. Hg in a group of eight preparations. The average for these eight filtration rates is 0.207 ± 0.026 gram per cent per minute. The standard deviation is about 13 per cent of the value of the mean. The error thus introduced by individual variation is minimal.

The curves in figure 2 show that the filtration rate in these preparations is a direct linear function of the physically determined osmotic pressure of the solutions. The equations of these two recti-linear curves are of the form:

$$y = E(B - h_o) \quad (3)$$

wherein E is a constant measuring the slope of the line, and B is a constant which includes the components of the driving force other than the osmotic pressure of the medium.

The second series of experiments were designed to measure the change in filtration rate when the perfusion pressure alone was varied. In figure 3, the relationship between perfusion pressure and filtration rate is presented, under the special conditions of perfusion with a medium having no colloid osmotic pressure. Therefore, the only component of the driving force involved is the hydrostatic pressure. Under these conditions the points obtained at the three higher pressures are satisfied by a straight line, while the two lower points fall below this line. Disregarding for the moment the lower points, the experimental results may be fitted by an equation of the form:

$$y = E(P - A) \quad (4)$$

where E is again a constant of slope, and A measures the decrease in pressure between the manometer and the filtration area.

Both equations 3 and 4 were derived empirically from the data obtained, and are special cases of the general law for filtration presented in equation 2. Equation 4, furthermore, fits the corresponding data taken from Krogh, Landis and Turner (2).

In figure 2 it will be seen that the value of E is less for the plasma perfusions than for the albumin perfusions, while the value of B in both equations is about the same. The constant E measures the change in the filtration rate for a unit change in driving force (in this case, for unit change in osmotic pressure) for the total surface area in 100 grams of tissue. For the albumin curve, E has the value of 0.00525 gram per cent per minute per centimeter H_2O change in osmotic pressure. For the plasma curve it is 0.0034. The difference in slope between the two lines indicates that at equivalent driving forces, the filtration rate per unit tissue is less with plasma than with albumin.

There are two explanations possible for this difference in their effectiveness: first, the permeability of the vascular wall may be altered directly by some constituent of the plasma which is not present in the purified albumin; the second, a vasoconstriction may result from perfusion with plasma which is not encountered during perfusion with albumin.

There is much experimental evidence to support the first explanation. Drinker (4) presented evidence that some component of serum has the ability to decrease capillary permeability. Sendery (7) showed that a pituitary hormone could alter the rate of edema formation or decrease the filtration rate in perfused limbs. The ability of adrenal cortical hormone to alter vascular permeability has been shown by Menkin (13), Freed and Lindner (14), and Hyman and Chambers (8). It is, therefore, possible that some chemical agent of this sort is active in altering the permeability of the membrane over which filtration must take place.

The hypothesis of a vaso-constrictor substance in the citrated plasma is also based on evidence from the literature. The existence of these vaso-constrictor substances in shed blood has often been reported. Bing (15) has reported on the effect of these substances on perfused organs. Reid and Bick (16) have studied some of the chemical characteristics of these substances. Such a vasoconstrictor substance could alter the filtration rate in two ways: the constriction

might be sufficient to eliminate completely certain of the capillary beds, thus altering the filtration area, or a partial constriction on the arterial side of the capillary bed would lead to an increased resistance in the arterioles, and a lower effective filtration pressure in the capillaries, changing the value of the constant a in equation 4.

The quantitative data obtained are compared directly with the value obtained by Krogh, Landis and Turner (2) in table 6. They found an increase of 0.0036 cc./100 cc. volume of arm for each centimeter of H_2O increase in osmotic pressure. This value is almost identical with the value of E obtained for plasma (0.0034) in the experiments reported in section A. The greater value for E obtained with albumin (0.00525) probably represents the filtration rate when none of the decrements discussed above for plasma are operative.

The intercepts for both the albumin and plasma curves are identical at a pressure of ca. 36 cm. H_2O . A solution having this physical osmotic pressure pre-

TABLE 6
Values of E . Change in filtration rate/100 cc. tissue

FOR 1 CM. H_2O CHANGE IN:	VENOUS PRESSURE	PERFUSION PRESSURE	OSMOTIC PRESSURE
Whole blood <i>in vivo</i> (Krogh, Landis & Turner, 1 c.).....	0.0024		0.0036
Plasma.....			0.0034
C.....		0.0013	
0.75C.....		0.00214	
0.50C.....		0.0030	
Bovine albumin.....		0.0030	0.00525
Saline.....		0.0036	

vents edema. Under these conditions the net driving force may be considered as 0. Since all the components of the driving force except the osmotic pressure were considered constant in both experiments, it is probable that the effective osmotic pressure of these media are equal when their physical osmotic pressures are the same. It is likely that none of the molecules of either of these two colloidal systems can escape across the vascular wall.

The curves obtained in the corresponding experiments with pectin and with gelatin (fig. 1) cannot be fitted directly by equation 3. The deviation from a linear relationship is marked in both these cases. Both of these colloids are poly-dispersed, and both have exceedingly great coefficients of asymmetry. Precisely how these characteristics of the material would intervene to alter the character of the curves in figure 1 is not apparent.

The intercept for the gelatin solution is exceedingly high. From the curves presented, it is apparent that under the same conditions of perfusion, a gelatin solution would have to have twice the osmotic pressure of an albumin solution to prevent edema. This fact is of practical value in the choice of an adequate blood substitute. One of the prime requirements for any blood substitute is

that it contributes significantly to the effective osmotic pressure. Therefore, the osmotic pressures of most substances which are proposed as blood substitutes are measured and these values presented as indicative of the efficacy of the various substances. The osmotic pressures measured in a physical system bear little relation to the effective osmotic pressures of the same colloid in the vascular system, unless the membrane used in the osmometer is identical with the vascular wall in its permeability characteristics. This caution is especially relevant since most of the substitutes considered are colloids of a highly poly-dispersed character.

In section B of the Experimental, a relationship between the rate of edema formation (outward filtration of fluid) and the perfusion pressure was established. Under the conditions of experiment 5 (fig. 3), it was pointed out that there is a linear relation between the pressure and edema rate at least for the higher pressures.

Gomez (17) presents evidence which suggests that deviations from Poiseuille's law at low pressures are associated with the characteristics of living arteries, and that non-living arteries do not show these abnormalities. He suggests that the low pressures may be insufficient to open the contracted arteries.

In experiments 6, 7, 8, 9 and 10 (on fig. 4) it was shown that there is a linear relationship between filtration rate as a function of perfusion pressure when the perfusion medium contains either albumin or plasma protein as its colloidal constituent.

The slopes of these lines (E of equation 4) are a measure of the change in rate of filtration per unit tissue for unit change in driving force. In the equation, however, the change in driving force is not directly measured by the change in the perfusion pressure. As has been pointed out above, the magnitude of the effective pressure in the capillary bed depends on both the arterial and venous pressures. In these experiments the venous pressure could be considered as zero, since the fluid had free passage out through the torn vena-cava. By the equation of Gomez (12), it can be shown that a unit change in arterial pressure *in vivo* should lead to a change of about $\frac{1}{4}$ of a unit in the mean capillary pressure, if the venous pressure is maintained constant, while a corresponding unit change in venous pressure would result in $\frac{5}{6}$ of a unit change in capillary pressure. Although this equation is probably not directly applicable to the preparations used, it suggests that the effective filtration pressure is a fraction of the perfusion pressure. The values of E should then be less than the true value of the change in filtration rate for unit change in capillary pressure, or for a corresponding unit change in venous pressure.

The value of E for variation in pressure when the colloid is bovine albumin, is apparently independent of the concentration of colloid, and is equal to 0.0030 gram per cent/min. for a 1 cm. H_2O change in perfusion pressure. In the case of the plasma proteins, however, the value of E increases with decreasing concentrations (see table 6). It will be noted that the E for undiluted plasma is considerable less than the value given by Krogh, Landis and Turner. This deviation is probably due to the fact that their value is given for unit change

in venous pressure, while the present value is for a unit change in perfusion pressure.

The variation in the value of E with altered concentration of plasma indicates some change in the system which depends on the concentration of plasma and which results in a changed filtration rate. The possible mechanisms whereby plasma might affect the filtration rate have been discussed above.

I wish to acknowledge my indebtedness to Prof. Robert Chambers for his guidance and assistance throughout this work.

CONCLUSIONS. First: For the preparations used, it has been possible to demonstrate a linear relationship between the colloid osmotic pressure of plasma and albumin solutions and the rate of filtration. The methods employed make it possible to obtain a value for the change in filtration rate for unit change in osmotic pressure. This value is greater for purified albumin than for reconstituted human plasma.

Second: There is a linear relationship between the perfusion pressure and filtration rate. The change in filtration rate for unit change in perfusion pressure likewise depends on the nature of the colloid used. For albumin, the change in rate of filtration per unit change in perfusion pressure is apparently independent of the concentration of the colloid. For human plasma, the corresponding change in filtration rate for unit change in perfusion pressure is dependent upon the concentration. It is least for the most concentrated solution.

Therefore, it is to be deduced that the filtration rate responds to changes in the driving force in the same way as with a physical ultra-filter membrane.

SUMMARY

1. A method for determining the filtration rate under controlled conditions is presented. This method depends on the continuous recording of the weight of a perfused preparation.

2. The relationship between the osmotic pressure of the perfusion medium and the filtration rate is established for four colloidal systems: albumin, reconstituted plasma, pectin and gelatin.

3. The relationship between the perfusion pressure and the filtration rate is established for three types of perfusion medium: colloid-free saline, albumin solutions, and several dilutions of reconstituted plasma.

4. The following conclusions may be drawn from the data presented:

a. There is an inverse linear relationship between the osmotic pressure of the perfusion fluid and the filtration rate when albumin or plasma is used.

b. There is a direct linear relationship between the perfusion pressure and the filtration rate.

c. The blood vessels of the preparation used can retain to a high degree molecules of the magnitude of albumin.

d. The blood vessels of the preparation used probably permit the passage of some fraction of the poly-dispersed gelatin system.

e. The effective osmotic pressure of a colloid in the vascular system is equal to the osmotic pressure determined in a physical osmometer only if the mem-

brane used in the osmometer and the vascular wall both retain the same fraction of the colloidal system.

REFERENCES

- (1) DRURY, A. N. AND N. W. JONES. *Heart*. 14: 55, 1927.
- (2) KROGH, A., E. M. LANDIS AND A. H. TURNER. *J. Clin. Investigation* 11: 63, 1932.
- (3) LANDIS, E. M. AND J. GIBBON. *J. Clin. Investigation* 12: 105, 1933.
- (4) DRINKER, C. K. *J. Physiol.* 63: 249, 1927.
- (5) ELLINGER, A. AND P. HEYMANN. *Arch. f. exper. Path. u. Pharmacol.* 90: 336, 1921.
- (6) DANIELLI, J. F. *J. Physiol.* 98: 109, 1940.
- (7) SENDEREY, S. *Rev. Soc. argent. biol.* 18: 244, 1942.
- (8) HYMAN, C. AND R. CHAMBERS. *Endocrinology* 32: 310, 1943.
- (9) IVY, A. C., H. GREENGARD, I. F. STEIN, F. S. GRODINS AND D. F. DUTTON. *Surg., Gynec. and Obstet.* 76: 85, 1943.
- (10) SIMMS, H. S., R. L. ZWEMER AND B. E. LOWENSTEIN. *J. Lab. and Clin. Med.* 28: 113, 1942.
- (11) BELLIS, C. J. *Surgery* 12: 251, 1942.
- (12) GOMEZ, D. M. Personal communication, 1944.
- (13) MENKIN, V. *This Journal* 129: 691, 1940.
- (14) FREED, S. C. AND E. LINDNER. *This Journal* 134: 258, 1941.
- (15) BING, R. J. *This Journal* 133: 21, 1941.
- (16) REID, G. AND M. BICK. *Austral. J. Exper. Biol. and Med. Sci.* 20: 33, 1942.
- (17) GOMEZ, D. M. *Hemodynamique et angiocinetique*, 1941.

MECHANISM OF THE EFFECT OF HYPERTHYROIDISM ON CARDIAC GLYCOGEN

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The cardiac manifestations of hyperthyroidism—hypertrophy, pathological changes, and chemical alterations—have been attributed by some investigators to a specific “toxic” action of the thyroid hormone, by others to purely physical and physiological phenomena; the proponents of neither viewpoint have made any serious attempt at an experimental approach to the problem’s resolution. This seems attainable most decisively by utilizing as test object a factor which can be quantitatively analyzed, which is altered significantly by hyperthyroidism, and which is not so altered by any disease or other uncontrollable process which might enter the picture. Such a test object is the glycogen content of the heart.

The earliest discoverable report that cardiac glycogen is diminished in hyperthyroidism appeared in a remark by Goodpasture (1) that he could discern no microscopically identifiable glycogen in the hearts of chloroformed, hyperthyroid rabbits. He did not make clear whether a similar loss was also to be found in hyperthyroid animals which had not been subjected to chloroform. Hoet and Marks (2) made the first definite statement, based on chemical analyses, that heart muscle was depleted of its glycogen content by thyroid feeding, and this conclusion has been repeatedly verified by many independent investigators.

The only other factor which has been found uniformly to elicit a loss of cardiac glycogen is a diminished oxygen supply. Thus, Evans (3) found that hypoxia depressed the heart’s concentration of glycogen in parallel with the extent to which oxygen was replaced by nitrogen. Similarly, it has been recognized for many years that tissues intended for glycogen analysis must be treated as rapidly as possible to stop glycogenolysis after arrest of the oxygen supply, and a major source of error has resulted from all-too-frequent disregard of this precaution.

That the glycogen depletion of the hyperthyroid heart is probably not accountable to avitaminosis B₁ is indicated by the repeated finding, most recently reported by Edlund and Holmgren (4), that severe thiamin deficiency does not lower cardiac glycogen. As a matter of fact, it is unlikely that the intensity and duration of hyperthyroidism induced in the present investigation is such as to cause any significant avitaminosis.

The decrease in heart glycogen resulting from the hyperthyroid state may be considered due either to a specific “toxic” effect or to a derangement in one or more physiological mechanisms as a result of the alteration of cardiac activity. If it is a consequence of increased activity of the heart, whether related to an augmented intensity of metabolism or work performance, or even to the increased

¹ Dissertation for Ph.D. degree in the Department of Physiology.

heart rate *per se*, then a similar loss of heart glycogen should be elicitable by chronically increasing cardiac activity through non-thyroid means. The present investigation is an attempt to stimulate the non-hyperthyroid heart, to depress activity of the hyperthyroid heart, and to analyze the effects on heart glycogen in the light of known physiological mechanisms.

METHODS. Male adult albino rats were used throughout. Although litter-mate controls were established in each experiment, it was not found necessary to separate the data, for the variations in control heart glycogens within any litter were no greater than those in the colony as a whole.

The basal ration consisted of Purina dog chow, *ad libitum*. This apparently led, at one stage, to a dietary deficiency resembling mild manganese-lack. It was soon eliminated by addition of weekly portions of lettuce, but not by carrots or cabbage. Subsequently, the lettuce supplement was replaced by the addition of about 10 ppm. of $MnCl_2$ to the drinking water. Under this regime, fertility was normal, fur and eyes appeared healthy, and growth was rapid, attaining a weight of well over 300 grams at six months.

During, and for at least three months before, the experimental period, the animals were housed in a constant temperature room at about 27°C.

Determination of heart glycogen. The method of preparing the heart for the glycogen analysis proved a major factor in obtaining consistent results. After a 24 to 36-hour fast, the unanesthetized rat was smartly transected somewhat above the level of the heart by a single cut with a large tinsmith's bench shear. Manual compression of the lower chest then exteriorized the heart, so that a single snip with scissors could drop it, preferably still attached by the pulmonary vessels to sufficient lung tissue to ensure flotation, into a container of vigorously boiling Ringer's solution. Time lapse between transection of the animal and destruction of glycolytic enzymes usually amounted to 1 to 3 seconds; actually, as many as 10 to 15 seconds could be lost in the operation without significant destruction of glycogen.

It was found necessary to use a fresh supply of boiling Ringer's solution for each heart.

The boiled heart was transferred to a shallow dish of Ringer's solution at room temperature, where it was carefully dissected free of all non-cardiac tissue. It was found advisable to eliminate the atria as well; otherwise, a variable amount of vascular tissue would have remained with the heart. The ventricles were cut into three or four portions, such that the chambers could be thoroughly freed from blood, and each segment was carefully blotted on filter paper, weighed on the torsion balance, and dropped into 2 cc. of 30 per cent KOH for the preliminary digestion.

The purification and hydrolysis of glycogen followed the procedure of Good, Kramer and Somogyi (5), and the glycogen hydrolysate was analyzed for glucose by Somogyi's (6) modification of the Shaffer-Hartmann method. The glycogen value was derived from the product of "glucose obtained" times the factor 0.927 and was in all instances converted to milligrams per cent.

As a result of the elimination of blood and non-cardiac tissues, the values obtained proved higher and more consistent than any to be found in the literature. The average, and standard deviation, on 20 normal rats was 659 ± 51 mgm. per cent. Extremes were 744 and 526, but since the latter value was the only one below 600 mgm. per cent, it may be assumed faulty, though not discarded. The individual values have been incorporated into graph *d*, figure 2.

Measurement of heart rate. Methods of counting the heart rate of the rat during forceful restraint, especially by needle electrodes, could not be expected to obtain even approxi-

mately basal values in these highly excitable subjects, and those involving palpation or auscultation appeared too prone to subjective variance. The procedure developed for the present investigation involved registration of the electrocardiogram from the surfaces of the animal's feet. At first, this was transcribed with the usual electrocardiograph, but, aside from the expensiveness of bromide paper and the time lost in developing the film, the machine was disadvantageous in that it failed to tap the heart's potential through the callouses which developed on the rat's feet as a result of friction against the false bottom of its cage. A change was made, therefore, to the apparatus devised by Gerard and his associates for the recording of brain waves. By this means, the impulse was picked up by contact electrodes, stepped up by Offner's (7) amplifier, and inscribed by the piezo-electrode crystograph of Offner and Gerard (8) on adding machine tape. Since the tape was moved by a constant speed motor, the heart rate could be measured with great accuracy by the use of dividers.

Construction of the animal holder is indicated in figure 1. Copper plates imbedded in the hard rubber floor served as pick-up electrodes, one for both forefeet and the other for the hind feet. Soldered to each plate was a long iron screw which pierced the floor and dipped into a mercury cup set into the shelf on which the cage rested. The potentials were thus led into the amplifier and crystograph. Animal, cage, and wiring were shielded from external interference by a copper mesh screen; such interference was frequently utilized as a time check by simply opening the screen door and taking a record of the 60-cycle hum. Neither electrode paste nor saline was required to facilitate transmission.

To simulate the rough floor to which rats apparently are partial, a square of copper screening was soldered to each of the copper plates. The sides and roof of the animal holder were formed by a single bent sheet of heavy celluloid. Slots cut into the celluloid provided apertures for the fastening screws at the sides of the cage floor and at the same time rendered the cage adjustable to animals ranging between 150 and 450 grams body weight.

The front gate was fashioned of heavy sheet metal with a fairly large copper screen window for ventilation. A 1½-inch overhang was bent into the upper part of the front gate to shade the animal's head. (This author finds no merit to the almost universal assumption that bright light induces rats to sleep; it probably irritates their eyes, which are minimally protected by pigment, and forces the animals to close them and to curl up for shade. It was observed, in fact, that the animals were quieter when the screen on the front gate was coated with flat black paint.)

A large slot was cut into the tail gate to allow protrusion of the tail and to avoid pinching the scrotum. It was also found advisable to use a small plate to close this slot in those instances, early in the training period, when a rat succeeded in turning around in the cage; without such a device, he was likely to gnaw at the edge of the tail gate.

With this set-up it was nevertheless found necessary to allot at least a month for training, before the recorded heart rates reached a plateau. This was done by placing about a dozen rats at a time into separate animal holders and simply leaving them for about half an hour every day.

The heart rate recorded for each run was the lowest one that was found in a ten-minute tracing, made after a ten-minute rest. Any bodily movement could be easily detected by irregular oscillations of the crystograph pen. It soon became clear, however, that physical activity was not the only factor causing variations in heart rate. In most subjects, neither the final "basal" rate of each record nor the rates obtained on different days jibed as completely as one might predict they would with the state of rest or activity. Since a spontaneous quickening of the pulse often anticipated a movement and since activity was in other instances neither accompanied nor followed by cardio-acceleration—sometimes, even, by a deceleration—it became patent that greater variation was induced by the psyche than by the soma.

Temperature changes likewise exercised an influence on the heart rate. On a few occasions, cold spells which were not anticipated and prepared for succeeded in lowering

the room temperature by several degrees. All heart rates taken during such periods were greatly elevated.

In assigning normal heart rates for comparison with those attained during the experimental period, each animal's graphic record of "basal" heart rates, covering a period of 3 to 6 weeks, was scanned and a "predictable" rate adopted. The resultant values, not truly basal for more than a small proportion of the 113 male rats, ranging between 200 and 370 grams body weight, which were used, averaged 4.4 ± 0.30 per second, or 264 ± 18 per minute.

Measurement of basal metabolism. The machine devised for determination of basal metabolism was of the closed-circuit type, measuring only the oxygen consumption. Water bath, spirometer box, and animal chamber were all constructed of sheet brass, which proved adequate for rapid equalization of temperature throughout the system. This temperature was maintained within narrow limits by means of a tubular model heating unit, controlled through a relay by a thermoregulator immersed in the water bath. The water was circulated by a noiseless electric stirrer. Effectiveness of the temperature control was checked by observing the time consumed in reaching constant volume after filling the system with oxygen; this process never required more than fifteen minutes. Once constant, the volume did not vary more than half a cubic centimeter during the interval between successive activations of the thermal element. Temperature within the animal chamber was kept at $28^{\circ}\text{C}.$, the temperature of thermic neutrality for the albino rat.

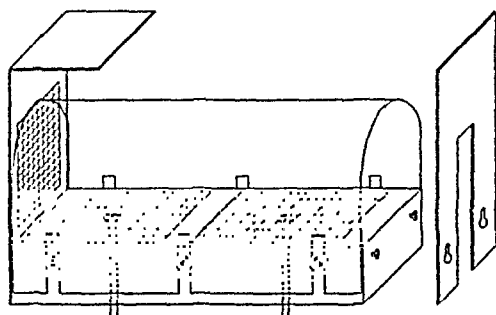


Fig. 1. Cage used in determining rat heart rate. Description in text. One-quarter scale.

The animal chamber was rendered airtight by the usual water seal. Its lid was provided with a large window made of two sheets of glass separated by an air space to limit condensation of water. The thermometer and a stopcock, to allow free movement of air into the system whenever the apparatus was opened, were fitted into the lid.

A false-bottom floor within the animal chamber was formed by the copper screen lid of a soda lime tray, whose bottom also was made of copper screening. This entire tray was elevated about an inch above the true floor of the animal chamber, leaving space for a pool of water below the CO_2 absorbent. Koehler (9) pointed out the advisability of maintaining a relatively high humidity within the chamber, partly to keep the soda lime at maximum absorptive efficiency and partly to induce the animal to relax. He considered that a vapor tension exceeding 75 per cent, however, was not thus conducive to rest. The present author, on the other hand, observed that the sedative action of humidity on rats varied directly with vapor tension and that a pool of water on the floor (99 per cent humidity at $28^{\circ}\text{C}.$) was most productive of rest. Under this regime, the animals almost immediately composed themselves for sleep, with but a small proportion of recalcitrants.

The spirometer bell was made of thin-walled brush copper, which is far less vulnerable than aluminum to attack by soda lime. Of the Krogh type, operating on the principle of a hinged box, it pivoted about jewel bearings, thus allowing complete freedom of movement without the shifting encountered in the cylinder type spirometer. The bell was counter-

weighted in such manner that, with the system opened to the outside, it descended slowly and without acceleration. At the end of the stainless steel tubular writing lever was attached a free-swinging capillary tubing writing point which exerted a constant pressure, determined by its own very slight weight, against the kymograph drum. Its tip was melted into a smooth ball to minimize frictional variation.

Use of these contrivances led to a high degree of sensitivity, so much so that the slightest movement of the rat communicated itself as gross oscillations to the kymogram. Such precision also made possible the measurement of oxygen consumption in five-minute periods. It was found that a sufficiently sensitive machine could be used for such short intervals, in actual fact gave lower and more constant results than any recording over longer periods.

In view of the fact that comparisons were to be made between the normal and experimental states in the same animal, all basal metabolism values were expressed as "cc./hr." Had body weight or surface area been used for reference, the loss of body fat with development of hyperthyroidism would have made the experimental figures inordinately high. For comparison with the data of other workers, however, the values were also converted for expression in the most customary terms. Of 132 determinations, during sleep, on 40 rats, the following equivalent averages were obtained:

686 cc./kgm./hr. (S. D., ± 45 cc.)

79.4 Cal./kgm./day

594 Cal./sq. m./day (S. = $kW^{2/3}$; $k = 9.1$)

These animals, all males, were 6 to 8 months old and ranged in weight from 267 to 370 grams (average, 315 grams).

In actual use, it was found that, although normal values were adequately constant, rarely varying as much as 10 per cent in any individual (sleeping state), determinations made upon hyperthyroid rats were highly unsatisfactory. Subjects which had previously gone to sleep within a few minutes of their entrance into the animal chamber were observed, when in the hyperthyroid state, making repeated attempts to compose themselves, but always failing. The basal metabolism determinations were therefore made under light nembutal anesthesia (40 mgm./kgm., ip.). This treatment depressed metabolism below that during deep sleep, in varying degree on different occasions, yet it gave a better comparison between normal and hyperthyroid values and was adopted as standard procedure.

Production of polycythemia. The finding of Waltner (10) that both the erythrocyte count and the hemoglobin value of rats' blood could be increased by cobalt feeding has been abundantly confirmed. This phenomenon was utilized in the present study by adding to the drinking water that amount of 10 per cent CoCl_2 which was calculated to afford each animal a daily intake of about half a milligram, in terms of the cobalt ion.

Since Orten *et al.* (11) showed a toxic effect of cobalt, and that it could be overcome by simultaneous feeding of manganese, an equal volume of 10 per cent MnCl_2 was used to supplement the cobalt, also by addition to the drinking water.

Production of anemia. The following method was developed for removal of large quantities of blood from rats. Under moderate nembutal anesthesia (50 mgm./kgm., ip.), the animal's tail was maximally vascularized by immersion in water at about 50°C . for about a minute. A two-inch length was then severed from the tail and the bleeding stump submerged in 5 per cent sodium citrate solution, preferably warm, to prevent coagulation. Usually, blood continued to spurt into the citrate for 10 minutes or more, and as much as $6\frac{1}{2}$ cc. could be removed thus from a 250-gram rat. Since the species has been estimated as possessing some $4\frac{1}{2}$ cc./100 grams body weight, this amounted to well over a 50 per cent depletion of its total blood volume.

By using a 25 cc. graduate cylinder previously charged with citrate to the 15 cc. mark, it was possible to control the quantity of blood removed by observing the level in the graduate and, at the desired time, arresting the hemorrhage by chilling under the cold water faucet.

Determination of hemoglobin. The method of Evelyn (12) was followed, except that the hemoglobin was oxygenated by shaking with 0.1 per cent Na_2CO_3 , rather than dilute NH_4OH , for a more stable oxyhemoglobin suspension. An Evelyn photoelectric colorimeter was used to determine the density of the blood- Na_2CO_3 mixture and the hemoglobin content calculated from the photometer reading.

EXPERIMENTAL RESULTS. In all experiments not otherwise specified, each rat underwent repeated determinations of heart rate and a single measurement, under nembutal anesthesia, of basal metabolic rate for establishment of its "predictable" values. After these standards were clearly attained, the particular experimental procedure was pursued for a period of 12 to 16 days, during which the changes in heart rate were followed and the terminal metabolic rate measured. Finally, the animal was sacrificed for analysis of cardiac glycogen, and comparisons were made with the normal and with the results of other procedures.

The alterations of basal metabolism obtained were taken as indicating the metabolic stimulation of the heart, on the basis of the observation by Dock and Lewis (13) that the rise in oxygen consumption of the rat heart-lung preparation paralleled that of the whole animal under thyroid administration.

Production of experimental hyperthyroidism. A moderate degree of hyperthyroidism was induced by feeding 25 mgm./100 grams/day of Armour's desiccated thyroid, blended into a small amount of salad dressing or grated carrot. Most animals consumed the mixture readily, especially with the latter vehicle; those few which refused were given the medication by force until the hyperthyroid state set in, after which they were nearly always so hungry as to require no further urging.

This single dosage level of thyroid substance, taken in all instances from a common bottle, exerted a highly inconstant effect on heart rate, metabolism and cardiac glycogen. The heart rates in 25 rats were accelerated by 5 to 50 per cent (average, 26 per cent); basal metabolism was increased in the 12 rats studied under nembutal anesthesia by 23 to 72 per cent (average, 51 per cent); and the hearts were depleted of their glycogen contents to a similarly variable extent (fig. 2).

Statistical handling of experimental and control data together did not prove advisable; the mere fact that all hyperthyroid animals varied in the same direction from the controls made several relations appear more real than closer scrutiny permitted. The correlation coefficient of the percentage increase in heart rate with the percentage increase in metabolism, determined according to the method of Fisher (14), gave an r value of 0.7799, corresponding to a probability of well under one chance in one hundred of the relation being fortuitous. But similar analysis of the experimental values alone obtained an r of -0.4649 , obviously without meaning, especially in view of its negative sign (see fig. 2a).

Correspondingly, trend lines, computed by the method of least squares, were distorted by the inclusion of control values. In figure 2, therefore, two such trends were constructed for each graph, one including control and experimental data (broken line), the other depending on experimental figures only (solid line).

The nearness with which those two trends coincided was taken as indicative of the innate relation between the factors involved.

As illustrated in figure 2, there was no close relation between the stimulation of heart rate and that of metabolism in hyperthyroidism (graph *a*), nor between the percentage increase in metabolic rate and the decrease of heart glycogen (graph *b*). On the other hand, 25 rats, including the 12 used in relating glycogen

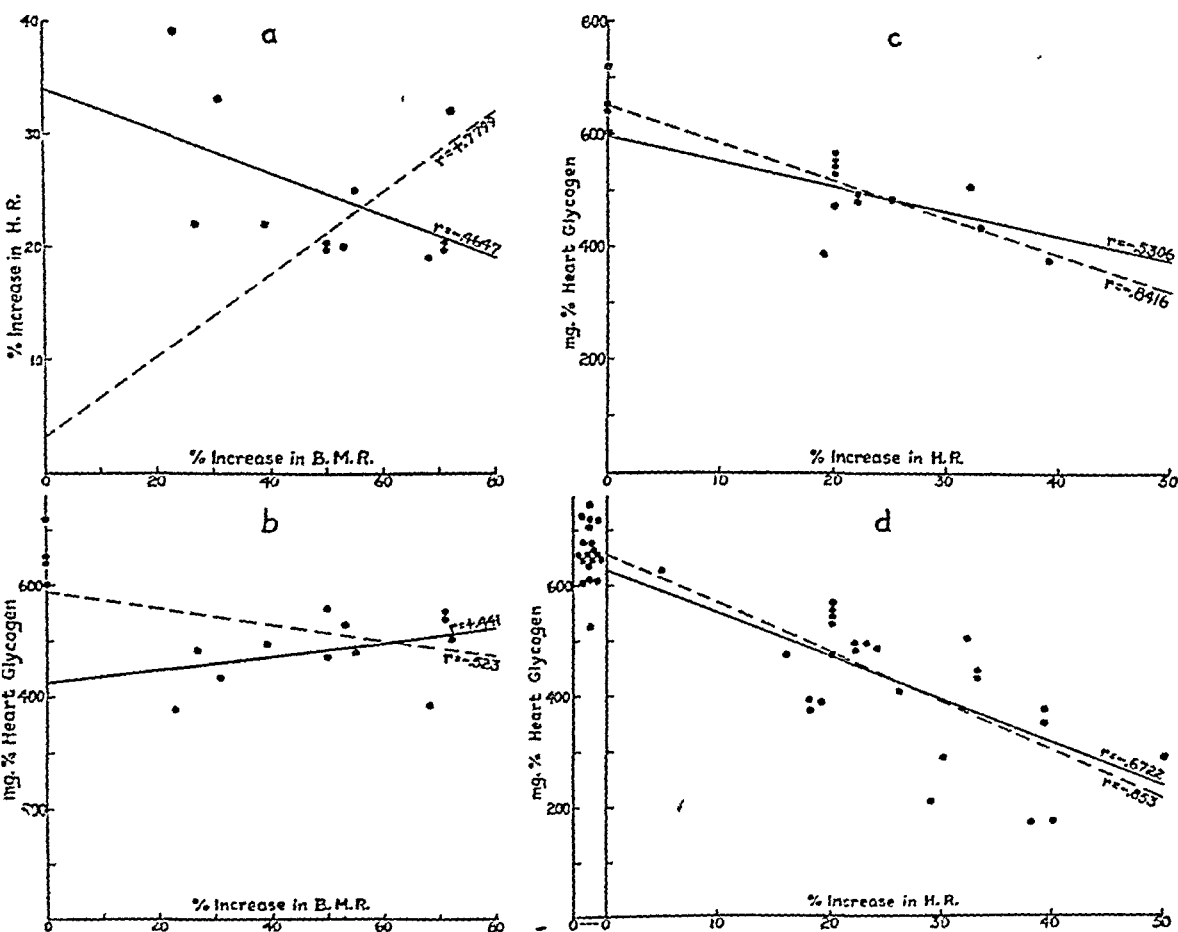


Fig. 2. Effect of hyperthyroidism. Solid trend lines are computed from experimental rats only; broken trend lines are from experimentals and controls, combined. Correlation coefficients based on experimentals only are placed at ends of solid trend lines; correlation coefficients derived from experimentals and controls are at ends of broken trend lines. *a*, relation of heart rate increase to metabolism increase; *b*, relation of heart glycogen to metabolism increase; *c*, relation of heart glycogen to heart rate increase (same individual rats as in *a* and *b*); *d*, relation of heart glycogen to heart rate increase (controls of entire series are placed at 0—0).

to metabolism, showed that the decrease in heart glycogen roughly but definitely paralleled the percentage increase in heart rate (graphs *c* and *d*).

Deceleration of hyperthyroid heart. Several attempts were made to depress the rate of the hyperthyroid heart to or toward normal. These included the intra-peritoneal injection, twice each day, of as much as 6 cc. (1.2 mgm.) of lanatoside

C², 0.3 mgm./100 grams of thevetin³, 0.02 mgm./100 grams of carbaminoylcholine chloride, and 15 mgm./100 grams of quinidine sulphate. In no instance was the pulse retarded; in some individuals, an even greater tachycardia was produced.

None of the data assembled on these animals was incorporated into any graph, since, in the face of the atypical effect on heart rate, their actions on other phases of cardiac physiology could not be deduced from the literature. However, the glycogen values obtained were such that they could have been inserted without distortion into the graph relating glycogen decrease to the degree of tachycardia (fig. 2*d*). That is to say, administration to hyperthyroid rats of none of these agents had any significant effect on either the tachycardia or the depression of heart glycogen.

Cardiac acceleration by non-thyroid means. Chilling. Subjection of rats to increased thermoregulatory demands was accomplished by depilation with saturated BaS of the entire body below the head. Some of the rats were subsequently placed in an environmental temperature of about 20°C., some in the refrigerator at 2°C. (all these animals died), and some were kept at 27°C.

According to the findings of Hamilton, Dresbach and Hamilton (15) and of many other investigators, a depression of body temperature and of heart rate, along with an elevation of metabolism, were expected to occur on thus chilling the animals. Actually, probably because no restraint was exercised upon the rats during these relatively prolonged experiments, an increase in heart rate obtained in the five hyperthyroid, normal and thyroidectomized rats which survived the several maneuvers.

Plotting the tachycardia thus produced against the terminal heart glycogen values gave the curve reproduced in figure 3*a*, to all appearances identical with that derived in hyperthyroidism alone; the trend line from figure 2*d* has been inscribed on this and other graphs for comparison. That the glycogenolytic effect was not exercised through a stimulation of thyroid activity was shown by 1, its occurrence in the thyroidectomized animals, and 2, the evidence of thyroid histology in the chilled normal and thyroid-treated animals. Hematoxylin-eosin sections from the chilled normal rats showed predominantly a flat acinar epithelium and colloid-filled alveoli, with a small proportion of partially empty alveoli having low cuboidal lining cells. The thyroid gland of the thyroid-fed rat which had been chilled exhibited only colloid-swollen acini surrounded by flat epithelium. Apparently, there was no stimulation of thyroid activity by the moderate chilling produced. The rectal temperature was reduced by about 1.5°F.

The completeness of thyroidectomy was vouched for by a definite fall in both heart rate and metabolism and by an absence of macroscopically identifiable thyroid tissue at autopsy.

Atropine. Atropine sulphate was administered by intraperitoneal injection of 10 mgm./100 grams, twice daily. This procedure was found to maintain the heart rate at levels approximating those obtained in the thyroid feeding experi-

² Lanatoside C (Cedilanid) was supplied by courtesy of Sandoz Chemical Works, Inc., New York.

³ Thevetin was supplied by courtesy of Eli Lilly & Co., Indianapolis.

ments, though the pulse probably began to regress toward normal during the hour or two preceding the morning injection. Since the dosage required was quite close to lethal, several animals died before completion of the course of injections; seven survived to the end.

The relation between cardiac acceleration and glycogen, expressed by the usual graphic method in figure 3b, roughly followed the same course as in the hyperthyroidism experiments.

Caffeine. As a means of stimulating the myocardium directly, caffeine citrate was given intraperitoneally in twice daily amounts of 8 to 15 mgm./100 grams. The smaller dosage was effective during the first few days of treatment, but development of tolerance by the animals required that the quantity be increased progressively. Maintenance of any constant degree of tachycardia was relatively unsuccessful and fluctuations therefore unavoidable.

As indicated in figure 3c, the depression of cardiac glycogen in the five rats which completed the experiment was clearly less than that which might have been expected from the levels of tachycardia developed. That difference might have been due to a simultaneous improvement of blood supply to the myocardium, resulting from the actions of caffeine in increasing general blood pressure and dilating the coronary vessels.

Ephedrine. Attempting to test the suspected cause of caffeine's difference from the other cardio-accelerators used, ephedrine sulphate was administered to one litter, alone or after production of hyperthyroidism. The maximum dose tolerated, 6 mgm./100 grams, ip., twice a day, produced relatively a slight speeding of the heart's action, followed after about six hours in most animals by a fall to, or even below, the control pulse level.

Of the five surviving rats, three were hyperthyroid. Two of these animals showed a decrease in heart rate, after the early rise, to somewhat below their hyperthyroid level, and their heart glycogens were 330 and 335 mgm. per cent, probably most nearly related to the degree of tachycardia they would have had with thyroid alone. The pulse rate of the other hyperthyroid individual was depressed to its control level, namely, the level before thyroid treatment was instituted; that animal's glycogen value was 523 mgm. per cent. Of the two non-hyperthyroid rats, one received a slight tachycardia from the ephedrine injections, while the other rat's heart rate was unchanged or slightly reduced; their heart glycogen concentrations were 403 and 692 mgm. per cent, respectively.

The consequences of ephedrine administration, thus, proved variable on both heart rate and heart glycogen, and no conclusions could be drawn from that experiment. The literature, too, has shown such a diversity; both an increase and a decrease in heart rate and blood pressure have been reported for unanesthetized animals by Chen and Schmidt (16).

Alterations in oxygen supply to the heart. The results obtained with caffeine administration, along with the well-known effect of anoxia in depressing heart glycogen (3), pointed to the possibility that the loss of glycogen in hyperthyroidism might be due to a hypoxia, depending on a relative anemia of the excessively

active heart. A method of testing this hypothesis was offered by studies on polycythemia and anemia.

Anemia. Five rats were bled by the method described, and their cardiac glycogen and hemoglobin values were determined five days later. The relation of heart glycogen to hemoglobin (fig. 4a) showed a decline in glycogen beginning at a hemoglobin level of 11 or 12 grams per cent and falling off rapidly thereafter.

The normal hemoglobin value, determined in six untreated rats, was 16.5 grams per cent; range, 15.4 to 17.4 grams per cent.

Polycythemia. Of a total of 23 cobalt-treated animals, 16 were rendered hyperthyroid and, with their 7 polycythemic controls, studied in the usual manner. The relation between heart rate increase and glycogen content (fig. 4b) showed a possible trend toward conservation of cardiac glycogen, especially at the higher

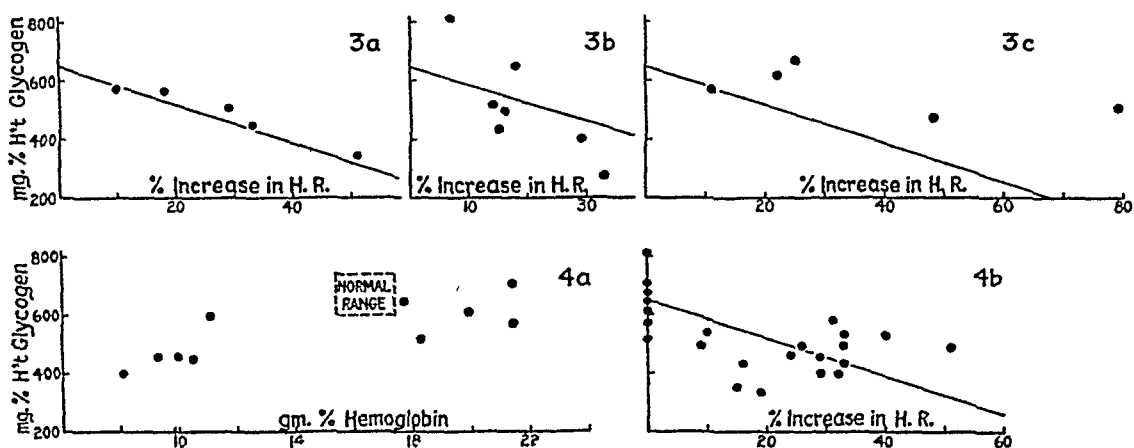


Fig. 3. Effect on heart glycogen of tachycardia produced by non-thyroid agents. Superimposed on each graph is the trend line obtained in figure 2d, relating heart glycogen to percentage increase in heart rate by thyroid treatment. a. Effect of chilling by depilation with barium sulphide. b. Effect of atropine. c. Effect of caffeine.

Fig. 4. Influence on heart glycogen of blood hemoglobin level. a. In non-hyperthyroid rats. Anemic rats at left, polycythemic at right, normal range as indicated. b. In polycythemic hyperthyroid rats. Polycythemic non-hyperthyroid controls are placed on ordinate guide line. Trend line from figure 2d is superimposed.

levels of tachycardia. The range of hemoglobin in 11 of these hyperthyroid polycythemic rats, the only ones on whom determinations were made, was 16.2 to 21.4 grams per cent.

The conclusiveness of this experiment is not overwhelming. Among those hyperthyroid polycythemic animals whose hemoglobin values were obtained, there was evinced no clear-cut protective trend; unfortunately, the four rats whose heart glycogens were highest above the expected were not among those whose hemoglobin levels could be determined. On the other hand, as indicated by the polycythemic control glycogens (fig. 4, a and b), the range of heart glycogen values in non-thyroid-treated animals was extended to limits of at least 811 and 516 mgm. per cent, implying a separate, variable influence of the cobalt treatment itself. Aside from this, there could be no assurance that the necessar-

ily increased viscosity of the polycythemic blood did not counterbalance any beneficial effect of the elevated oxygen carrying power.

Relative depletion of right and left ventricles. Another avenue of attack was offered by the fact that coronary blood flow to the musculature of the left ventricle is interfered with during systole. The deeper branches of these arteries have been demonstrated to be subjected to high external pressure by the contracting heart wall, thus impeding flow and relegating it mainly to the diastolic phase of the cardiac cycle. It has also been amply shown that an acceleration of the heart occurs primarily at the expense of the recovery period, so that any abbreviation in the cardiac cycle takes place as an encroachment on diastole, affecting systole but little. Hence, the proportion of the cycle during which there is free coronary flow to the subendocardial portion of the left ventricle must be less in a rapid than in a slow heart.

On these grounds, it seemed likely that, if the loss of heart glycogen during prolonged tachycardia is accountable to deficient blood flow—i.e., relative anoxia—to the heart musculature, the depletion would be correspondingly greater in the left ventricle. Comparative determinations were therefore attempted on the right and left ventricles, separately, in hyperthyroid rats. The glycogen analysis, however, proved far less reliable with the small amounts of tissue here involved, especially in the instance of the right ventricle, than those performed in the other experiments. The time elapsing between opening of the chest and immersion of the heart in boiling Ringer's solution became extremely important. Most consistent results, though still far from being completely satisfactory, were obtained when this "removal time" was held under two seconds and when the amount of left ventricle used for analysis was limited to less than twice the weight of the right ventricle. The values obtained on the 16 normal and 19 hyperthyroid hearts which accorded with these requirements (table 1) showed a generally higher glycogen concentration in the left ventricle in normal hearts and in the right ventricle in hyperthyroidism. The apparent loss of glycogen, upon moderate thyroid medication, by the right ventricle was 13 per cent, by the left ventricle 18 per cent, amounting to a 37 per cent greater loss by the left ventricle than by the right.

This evidence apparently favors the theory that the glycogen loss is attributable to a relative ischemia, exaggerated in the left ventricle by the dynamics of coronary circulation in that chamber. However, statistical analysis of these data showed that the significance of the difference between the means of control and experimental glycogens in the right ventricle had a t value of 3.04, of the left ventricle 5.95. The difference, though suggestive, is thus not highly significant.

DISCUSSION AND CONCLUSIONS. The observation that hyperthyroidism, chilling and atropinization all decrease cardiac glycogen, depending directly on the degree of tachycardia, seems to indicate that that effect is directly and causally associated with the increase in cardiac activity. The influence of cooling and of atropine are probably alike exerted predominantly or entirely through the nervous system, giving as nearly a pure acceleration as could be established in chronic animals, yet their effects on the glycogen level are as great as that of thyroid feeding.

These observations indicate that the glycogen depletion, at least, of the so-called "thyroid" heart is not due to any specific action peculiar to the thyroid hormone. Were it to be found possible to prevent or inhibit the tachycardia of hyperthyroidism, a more crucial test of the apparent relation to cardiac activity would be feasible.

The lessened glycogen depletion obtained with caffeine administration implies a major rôle on the part of some additional factor. That this factor may be one of blood supply to the heart wall is suggested by caffeine's action of increasing

TABLE 1
Comparative depletion of glycogen in right and left ventricles

CONTROL			HYPERTHYROID		
Right ventricle	Left ventricle	R.V. minus L.V.	Right ventricle	Left ventricle	R.V. minus L.V.
357	498	-141	466	441	+25
709	645	+64	580	521	+59
528	556	-28	425	480	-55
518	602	-84	426	424	+2(=)
579	567	+12	423	523	-100
638	650	-12	411	448	-37
640	669	-29	496	472	+24
436	473	-37	541	508	+33
537	535	+2(=)	482	465	+17
470	518	-48	451	568	-117
643	683	-40	519	442	+77
506	543	-37	602	528	+74
617	567	+50	478	443	+35
590	590	0(=)	496	424	+72
581	591	-10	465	439	+26
568	550	+18	557	511	+36
			459	456	+3(=)
			468	471	-3(=)
			435	450	-15
Av.: 558	577	-19	Av.: 483	474	+9

	R.V. > L.V.	R.V. < L.V.	R.V. = L.V.
Control.....	4	10	2
Hyperthyroid.....	12	5	3

cardiac circulation by raising the general blood pressure and dilating the coronary arterioles. Partial support for this explanation derives from the loss of heart glycogen brought about solely by severe anemia, and, contrariwise, by the tendency toward protection by polycythemia.

The theory may be advanced, quite tentatively, that a reduction of glycogen stores in the heart is consequent upon an ischemia to the myocardium. An increase in cardiac activity, beyond the limit of a parallel increase in blood supply under the particular conditions, must result in a relative anoxia. In the tachycardia of hyperthyroidism, some reflex and chemically stimulated adjustment of

circulation is doubtless made, but it is quite possible that the coronary dilatation which does occur is able to obtain insufficient additional blood flow in the face of more forceful demands by other hypermetabolizing tissues. The major driving force for coronary blood flow—aortic pressure—is not significantly augmented in hyperthyroidism.

This concept of an ischemic etiology of the glycogen depletion accords well with the ideas advanced by Goodpasture (1) and by Simonds and Brandes (17) in their attempts to explain the microscopic appearance of the "thyroid" heart. Similar cardiac histopathology to that claimed specific for hyperthyroidism was described by Thorel (18) for patients with coronary insufficiency and by Schmidt-mann (19) and Pfeleiderer (20) for sections prepared from the hearts of rats, guinea pigs and rabbits in which coronary disease had been experimentally produced. The fatty and hyaline degeneration, round cell infiltration and scar tissue formation, frequently perivascular, which have been described as characteristic of thyroid intoxication are precisely what one should expect from myocardial ischemia.

A definitive statement cannot, however, be made until actual studies of coronary arterio-venous oxygen differences have been carried out. Certainly the finding of Essex *et al.* (21) that the coronary flow may be increased in hyperthyroid dogs by a maximum of about 200 per cent does not support such a concept; it would strongly controvert this idea were that maximum maintained over a period of days or weeks.

The increased rate and work of the rapid heart are coupled with a necessarily abbreviated recovery time between beats, as well as a diminished period during which circulation is at its maximum, and these forces may well set into motion a vicious cycle. Whether they actually do so must be determined by direct experimental analyses, preferably simultaneously, of these several factors.

The author wishes to express his gratitude to Prof. A. B. Luckhardt and to other members of the Department of Physiology, as well as individuals in other departments of the University of Chicago, for advice and encouragement during the conduct of this investigation.

SUMMARY

1. Methods were developed for accurate and consistent determination of heart rate, oxygen consumption and cardiac glycogen in the white rat. A technique was also devised for the removal of large amounts of blood from the rat's tail.

2. Standard "predictable" levels of heart rate and basal metabolic rate were established in 110 rats. After approximately two weeks' experimental manipulation, cardiac glycogen contents were determined and compared, in relation to the changes produced in heart rate and metabolic rate, with normal controls and with each other.

3. Moderate hyperthyroidism was found to deplete heart glycogen to an extent directly related to the increase in heart rate but not closely related to the metabolic stimulation.

4. Atropine and chilling (by depilation) decreased heart glycogen, in relation to the tachycardia produced, to a degree similar to that obtained in hyperthyroidism.

5. Caffeine caused a smaller loss of heart glycogen, with reference to the stimulation of heart rate, than would have been predicted from the effects of thyroid, atropine and chilling. This might have resulted from the increased blood pressure and the coronary dilatation characteristic of caffeine medication.

6. Primary anemia produced a decrease in heart glycogen roughly correlated with the decline in hemoglobin content of the blood. Cobalt polycythemia seemed, to a slight extent, to protect the hyperthyroid heart from loss of its glycogen stores.

7. The decrease in heart glycogen of 19 hyperthyroid rats was participated in by both the left and right ventricles. Loss by the left ventricle was greater by an average of 37 per cent, but the variability of results, due to technical difficulties, renders this difference not statistically as significant as might be desired.

It is concluded that the decrease in heart glycogen by hyperthyroidism is not due to any specific "toxic" influence of the thyroid hormone. The suggestion is tentatively offered that the glycogenolytic action is exerted through a relative ischemia caused directly and indirectly by the increased cardiac activity.

REFERENCES

- (1) GOODPASTURE, E. W. *J. Exper. Med.* **34**: 407, 1921.
- (2) HOET, J. P. AND H. P. MARKS. *Proc. Roy. Soc., London, s.B.* **100**: 72, 1926.
- (3) EVANS, G. *This Journal* **110**: 273, 1934.
- (4) EDLUND, Y. AND H. HOLMGREN. *Ztschr. f. d. ges. exper. Med.* **109**: 11, 1941.
- (5) GOOD, C. A., N. KRAMER AND M. SOMOGYI. *J. Biol. Chem.* **100**: 485, 1933.
- (6) SOMOGYI, M. *J. Biol. Chem.* **117**: 771, 1937.
- (7) OFFNER, F. *Rev. of Sci. Instru.* **8**: 20, 1937.
- (8) OFFNER, F. AND R. W. GERARD. *Science* **84**: 209, 1936.
- (9) KOEHLER, A. E. *J. Biol. Chem.* **95**: 67, 1932.
- (10) WALTNER, K. *Arch. f. exp. Path. u. Pharmacol.* **141**: 123, 1929.
- (11) ORTEN, J. M., F. A. UNDERHILL, E. R. MUGRAGE AND R. C. LEWIS. *J. Biol. Chem.* **99**: 465, 1932.
- (12) EVELYN, K. *J. Biol. Chem.* **115**: 63, 1936 (Method detailed in EVELYN photoelectric colorimeter: Notes on operation, p. 34. Rubicon Co., Philadelphia).
- (13) DOCK, W. AND J. K. LEWIS. *J. Physiol.* **74**: 401, 1932.
- (14) FISHER, R. A. *Statistical methods for research workers.* 1936. 6th ed., Oliver and Boyd, Edinburgh.
- (15) HAMILTON, J. B., M. DRESBACH AND R. S. HAMILTON. *This Journal* **118**: 71, 1937.
- (16) CHEN, K. K. AND C. F. SCHMIDT. *Ephedrine and related substances.* 1930. Williams & Wilkins, Baltimore.
- (17) SIMONDS, J. P. AND W. W. BRANDES. *Arch. Path.* **9**: 445, 1930.
- (18) THOREL, C. *Ergebn. d. allg. Path.* **17**(2): 90, 1915.
- (19) SCHMIDTMANN, M. *Centralbl. f. allg. Path.* **54**: 200, 1932.
- (20) PFLEIDERER, E. *Virchow's Arch. f. path. Anat.* **284**: 154, 1932.
- (21) ESSEX, H. E., J. F. HERRICK, E. J. BALDES AND F. C. MANN. *This Journal* **117**: 271, 1936.

THE OXYGEN TENSION OF ARTERIAL BLOOD AND ALVEOLAR AIR IN NORMAL HUMAN SUBJECTS^{1,2}

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There is no agreement in the experimental literature or in standard reference works in physiology as to what constitutes normal human arterial oxygen tension. Douglas and Haldane in 1912 (10) reported tensions ranging from 91 to 103 mm. Hg (average 97 in 5 expts.); their subjects were not breathing room air, but 19.54 to 21.78 per cent oxygen. Barcroft (1, 2) published one determination in 1921 and another in 1923; the values obtained were 99 and 100 mm. Hg. In 1930 Bock et al. (6) found normal arterial pO_2 to be 63 to 78 mm. Hg in 4 subjects (average 73). In 1936 Hick (12) recorded tensions of 74 to 88 mm. Hg in 9 subjects (average 79) and finally in 1942 Cullen and Cook (9) performed 25 experiments upon normal men and obtained arterial oxygen tensions varying from 67 to 79 (average 72). Illustrative of figures listed in current reference works are the following: 100 (5), 90 (20), 80 (18), and 72 mm. Hg oxygen tension (4). In addition to these discrepancies it is also variously reported that the difference between alveolar and arterial oxygen tensions is 1 (1) to 25 (6) mm. Hg.

It therefore seemed of the utmost importance to reinvestigate these two problems simultaneously with methods as simple and as accurate as possible. The methods used to date in measuring arterial oxygen tension (pO_2) have varied. They include an aerotonometer (1, 2, 12), an improved CO method (10), and indirect evidence derived from oxygen dissociation curves (6, 9). We have used a direct method based upon the technique of Barcroft and Naga-hashi (1).

METHODS. Since we believe that the chief reasons for the discrepancies noted above lie in the methods used for determining arterial O_2 tension we are describing our method in detail: 15 ml. of arterial blood is drawn by brachial artery puncture into a syringe, the dead space of which was previously filled with

¹ A preliminary report of these investigations was made before the Philadelphia Physiological Society on May 16, 1944 (Am. J. Med. Sc. 208: 135, 1944). Since that time an improved technic has been devised and consequently none of the 13 determinations reported at that time has been included in this report.

² The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Pennsylvania. Financial support was also received from the National Committee for Mental Hygiene from funds granted by the Committee on Research in Dementia Praecox founded by the Supreme Council, 33° Scottish Rite, Northern Masonic Jurisdiction, U. S. A.

heparin. The blood is immediately transferred to a tonometer (fig. 1A) containing mercury and 30 cu. mm. of room or alveolar air; most of the mercury is displaced by blood (fig. 1B) and the tonometer containing blood, gas and a small amount of mercury at atmospheric pressure is then rotated in a water bath at 38°C. for 10 min. During this period equalization of oxygen tension occurs between gas and blood at a pressure very close to the initial blood tension (since the ratio of blood volume to gas volume is 500:1). Room air was used in the preliminary controlled equilibrations in order to determine the length of time required to insure equalization of the tensions; in all other experiments alveolar air was used so that equilibration was achieved with a minimum of gas exchange. At the end of this time, a small glass pipette with a 2 mm. internal

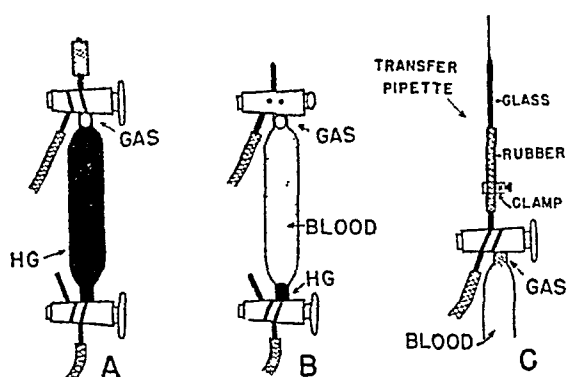


Fig. 1

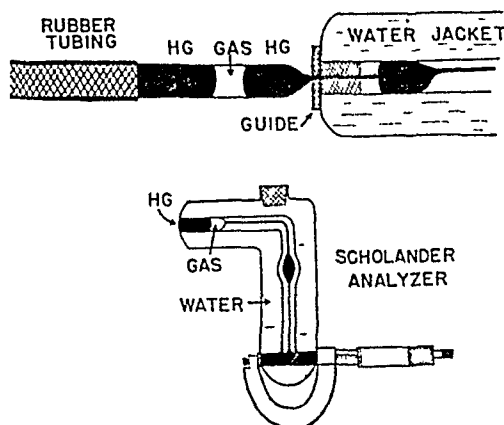


Fig. 2

Fig. 1. Tonometer and transfer pipette used in analyses (see text).

A. Tonometer, containing mercury and 20-30 cu. mm. of air or alveolar air, ready for transfer of arterial blood from syringe. Mercury leveling bulb not shown.

B. Tonometer after transfer of blood. Stopcocks are then closed, tubing from mercury leveling bulb is removed, and the tonometer is then rotated in water bath at 38°C. for 10 min.

C. Tonometer with transfer pipette attached.

Fig. 2. Above: The gas bubble, trapped between two columns of mercury, is being transferred to the Scholander micro gas analyzer (greatly magnified).

Below: The gas bubble, again between two columns of mercury, is ready to be analyzed. Note completely water jacketed Scholander micro gas analyzer.

diameter (fig. 1C) is attached to the tonometer by rubber tubing with a similar bore and the pipette is completely filled with mercury from the tonometer side arm. Great care is taken to dislodge any air bubbles which might be trapped at the rubber-tonometer or rubber-glass pipette junctions. The gas bubble is then transferred to the pipette from the tonometer, and more mercury is run into the pipette from the side arm; the gas bubble to be analyzed is thus trapped in the glass transfer pipette between two columns of mercury. The pipette tubing is then clamped and the pipette removed from the tonometer. Since the gas bubble usually becomes a fine froth during the equilibration, the last few millimeters of the pipette tubing is moistened with a very small amount of caprylic alcohol before the transfer is begun; in this way, clean gas bubbles free from contamination with blood are obtained.

The gas bubble is then transferred to a completely water jacketed Scholander micro gas analyzer (21) (fig. 2) and the oxygen percentage determined. The

arterial pO_2 is calculated according to the formula
$$pO_2 = \frac{(B-47) \times \% O_2}{100}$$

(It was found unnecessary to estimate total gas tension in the gas bubble since it proved to be equal to atmospheric pressure when equilibration was carried out with normal arterial blood. If pO_2 measurements were made upon venous blood or upon arterial blood with low pO_2 due to pulmonary pathology with failure of diffusion, an error would be introduced unless total tensions were first measured.) We have been unable to duplicate Scholander's results for CO_2 analyses, our figures always being lower than expected when microanalyses were performed upon samples of gas previously analyzed in the Haldane apparatus; consequently a small correction must be made for shrinkage in the initial gas volume due to loss of CO_2 through the manometer fluid of the microanalyzer. In the time required for the initial reading of total volume, shrinkage of the bubble occurs when the gas is a CO_2 -air mixture but no change occurs when the gas is a 12 to 14 per cent O_2 in 88 to 86 per cent N_2 mixture; consequently the assumption that the shrinkage of the gas bubble is entirely due to CO_2 loss is justified. This correction is made as follows: The pO_2 found is multiplied by 100 per cent less the difference between the subject's alveolar CO_2 per cent and the CO_2 per cent measured by the Scholander analyzer.

Scholander's technique has been modified also in respect to the solutions used; we employed N/1 KOH for CO_2 absorption, sodium hydrosulfite-sodium anthraquinone beta sulfonate (10:1) 2.2 grams in 10 cc. of N/1 KOH for O_2 absorption, N/1 H_2SO_4 as wash solution and 18 per cent Na_2SO_4 in 2 per cent Tergitol as the manometer fluid (the vapor tensions of these solutions vary less than 1 mm. Hg as measured in the Van Slyke manometric apparatus).

In table 1 are presented data showing the accuracy of the method as described for determining pO_2 in room air, in low oxygen mixtures and in gas bubbles equilibrated with bloods of known gas tensions. (The latter were prepared by equilibrating 25 ml. of blood with 1100 ml. of a known gas mixture (Haldane analysis) in a tonometer for 60 min.) It can be seen that blood pO_2 in this range may be measured by this direct method far more accurately than by indirect methods; the average of 11 determinations was -0.2 mm. Hg and the standard deviation was 2.7 mm. Hg.

Alveolar air samples were obtained at the end of inspiration and at the end of expiration by having the subject exhale rapidly and forcefully through a tube 4 feet long and 1 inch in diameter; samples were collected in a Bailey bottle attached to a side arm close to the mouth piece. Analyses were performed in the Haldane-Henderson apparatus.

A complete experiment was performed as follows: The subject lay in the semi-recumbent position in bed for 15 minutes. The skin and tissue overlying the brachial artery were infiltrated with 0.5 per cent procaine. An end-inspiratory alveolar air sample was collected. The arterial blood sample was

collected immediately thereafter; in no instance did the subject feel the needle enter the artery. Since no pull was exerted upon the plunger of the syringe, a period of about 1-2 minutes was required for collection of the blood sample; during the last 10 seconds of the collection an end-expiratory alveolar air sample was obtained. In some instances two or three end-inspiratory and end-expiratory samples were obtained. Subject 7 was unable to give satisfactory alveolar air samples.

RESULTS. Measurements have been made upon 13 healthy medical and pre-medical students between the ages of 17 and 24; all were male except subject no. 2. The average arterial pO₂ was 97.1 and the average alveolar end-expira-

TABLE 1

Accuracy of method in analyses of room air, of low oxygen mixtures, and of gases equilibrated with blood of known gas tensions

A ROOM AIR (pO ₂ = 159.0)		B LOW O ₂ MIXTURES			C BLOOD EQUILIBRATIONS		
pO ₂	Error (mm. Hg)	Actual pO ₂ (Haldane)	pO ₂ (Scholander)	Error	Actual pO ₂ (Haldane)	pO ₂ (Scholander)	Error
158.6	-0.4	108.9	107.9	-1.0	112.0	116.5	4.5
160.6	1.6	108.9	108.4	-0.5	118.7	116.0	-2.7
158.3	-0.7	110.2	109.8	-0.4	118.7	116.5	-2.2
160.0	1.0	110.2	109.7	-0.5	116.5	113.5	-3.0
158.1	-0.9	110.2	109.9	-0.3	119.2	119.4	0.2
158.4	-0.6	110.2	111.4	1.2	103.5	102.6	-0.9
158.5	-0.5	110.2	112.0	1.8	103.5	99.5	-4.0
159.0	0.0	110.2	109.7	-0.5	84.0	84.3	0.3
		110.2	110.3	0.1	90.2	92.9	2.7
Average	-0.1	110.2	109.5	-0.7	88.4	90.6	2.2
		110.2	111.0	0.8	88.1	89.4	1.3
		108.6	109.6	1.0			
		108.6	110.6	2.0		Average	-0.2
		108.6	108.6	0.0		Standard	2.7
		108.6	110.4	1.8		deviation	
			Average	0.3			

tory pO₂ was 97.4 mm. Hg; in 5 subjects the arterial and alveolar pO₂ differed by less than 1 mm. Hg (table 2). It is apparent upon the basis of these measurements that no difference exists between the pO₂ of "end-expiratory" alveolar air and arterial blood. It is also important to note that the variations in arterial blood pO₂ were less than those in alveolar air: 93-100.6 for the former and 86.2-104.4 for the latter. It appears that the arterial pO₂ determinations are actually more accurate and consistent than alveolar air determinations. This may be ascribed to two reasons: 1, the arterial blood collections require no co-operation upon the part of the subject while the alveolar air collections involve a high degree of co-ordination, timing and co-operation by the subject. 2. Since each blood sample was collected over a period of 1 to 2 minutes, it represented the

average pO_2 to which it had been exposed in the lungs during 10 to 30 inspirations and expirations; the alveolar air sample, on the other hand, represented only a spot determination during this period.

The end-inspiratory alveolar pO_2 figures are included for the sake of completeness though it must be pointed out that these too are spot samples and are not representative of alveolar air but rather samples containing the maximum pO_2 to which the venous blood is exposed during any respiratory cycle. Neither do the averages of end-inspiratory and end-expiratory samples represent a true average alveolar air since the peak of inspiration occupies a very small fraction of the total respiratory cycle. In the absence of an integration of the inspiratory and expiratory curves, it is highly probable that end-expiratory samples come closest to being "average" alveolar air samples under resting conditions.

TABLE 2

Comparison of oxygen tension in arterial blood and alveolar air in normal human resting subjects

SUBJECT	ART. pO_2	ALVEOLAR pO_2 END. EXP.	ALVEOLAR pO_2 END. INSP.	AVERAGE END. EXP. END. INSP.
1) W.K.	95.1	95.5	99.2	97.3
2) M.D.	100.1	104.4	112.3	108.3
3) W.P.	95.7	95.8	99.5	97.6
4) J.K.	98.1	86.2	105.4	95.8
5) R.J.	96.0	92.8	106.1	99.4
6) A.P.	97.5	97.1	105.3	101.2
7) R.A.J.	100.1			
8) D.F.	96.4	94.3	103.1	98.7
9) J.A.	93.0	101.7	99.4	100.5
10) D.M.	93.5	98.6	105.2	101.9
11) B.R.	100.6	101.3	99.4	100.3
12) I.P.	96.8	101.6	100.9	101.2
13) W.D.	99.5	99.8	105.2	102.5
Average	97.1	97.4	103.4	100.4

DISCUSSION. Neither the CO method of Haldane (10), nor the aerotonometer technique of Barcroft (1) has been used to any degree during the past 20 years. Instead reliance has been placed on indirect determinations. These involve measurements of oxygen saturation and pH of arterial blood, and then reading the pO_2 from the oxygen dissociation curve of human blood. It cannot be too strongly emphasized however than this method is wholly unsatisfactory when the O_2 saturation is between 92 and 98 per cent (the flat portion of the O_2 dissociation curve). A consideration of two of the most carefully constructed O_2 dissociation curves in the literature will illustrate this point (7). The blood of A.V.B. equilibrated with 70 mm. pO_2 was 94.5 per cent saturated, equilibrated with 80 mm. pO_2 it was 96 per cent saturated, equilibrated with 90 mm. pO_2 it was 97.0 per cent saturated and equilibrated with 100 mm. pO_2 it was 98 per cent saturated. Similar figures were recorded for G.S.A.: at 70 mm. pO_2 95.5

per cent saturation, at 80 mm. pO₂ 97 per cent saturation, at 90 mm. pO₂ 97.5 per cent saturation and at 100 mm. pO₂ 98 per cent saturation. Thus a change in oxygen tension from 70 to 100 mm. Hg will increase the O₂ saturation only from 94.5 to 98 per cent or from 95.5 per cent to 98 per cent. Since duplicate determinations of O₂ saturation (by experienced technicians, using the Van Slyke-Neil method¹ (22)) may vary 0.5 to 1.2 per cent, it is evident that the computation of arterial pO₂ by reading from the flat portion of the oxygen dissociation curve may involve an error of as much as 20 mm. Hg. This error may be increased when the oxygen dissociation curve used is one constructed from blood of a subject other than the one whose tension is being calculated.

Furthermore it has recently been shown by Boothby (8) (using the oximeter), by Roughton et al. (19) (by an analysis of the errors inherent in determinations of O₂ saturation by the Van Slyke-Neil method) and by Drabkin et al. (11) (using a spectrophotometric technique) that normal human arterial blood is about 97.5 to 98.5 per cent saturated with oxygen rather than 94 to 96 per cent.

The errors involved in computing arterial pO₂ from the flat portion of the dissociation curve and those inherent in the actual gasometric analyses for O₂ saturation are probably responsible in great part for the low arterial oxygen tensions obtained by most investigators.³ If normal arterial O₂ saturation figures of 97.5 to 98.5 per cent be accepted as correct (8) (19) (11), then reference to dissociation curves of A.V.B. and G.S.A. (7) shows that the expected arterial pO₂ would be 100 mm. Hg. Our average figure is 97.1 mm. Hg.

Our data indicate also that there is practically no difference between "end-expiratory" alveolar air and arterial blood oxygen tensions, yet, as has already been noted, many investigators report differences ranging from 1 to 25 mm. Hg. These differences occur chiefly in studies upon subjects breathing room air at sea level. When the problem has been investigated at high altitudes or in individuals breathing low oxygen mixtures (2, 6) little or no difference between alveolar and arterial tensions has been noted. Such results can be explained largely on a basis similar to that outlined above: when the oxygen saturation is below 90 per cent (due to breathing air with a low oxygen tension) the slope of the O₂ dissociation curve is steep and a large change in O₂ saturation is brought about by a small change in pO₂; consequently estimates of arterial pO₂ from the O₂ dissociation curve (after arterial O₂ saturation and pH have been measured) are accurate in these lower ranges though highly unsatisfactory in the normal range.

It should also be emphasized that our average figure for arterial pO₂ of 97.1 mm. Hg applies only to the conditions of our experiments—resting, semi-recumbent normal subjects between the ages of 17–24. Conditions which increase the

³ The low arterial pO₂ reported by Hick (12) (using an aerotonometer and a Krogh microanalyzer) can be explained only on the assumption that experimental errors occurred; this is probable since subject C (12, p. 536), having an arterial saturation of 94 per cent and an arterial pO₂ of 87 mm. Hg while breathing room air, maintained an arterial pO₂ of 87 mm. Hg even when inhaling a mixture low enough in O₂ to reduce his saturation to 78 per cent.

depth of respiration will obviously increase the arterial pO_2 and conditions which decrease the depth of breathing will decrease the arterial pO_2 . It thus becomes apparent that a "normal" arterial tension cannot be an absolute figure but depends upon the conditions of the experiment. In similar fashion differences between alveolar and arterial oxygen tensions must be critically examined, with particular emphasis on the degree and efficiency of respiratory activity of the subject and upon the time of collection of alveolar air samples. Thus Krogh (15) using an accurate aerotonometer, found that the arterial pO_2 of rabbits was 5 to 25 mm. Hg lower than the alveolar pO_2 ; in the light of our present experiments, this difference was probably due in large part to the anesthetic used with consequent hypoventilation, atelectasis or inhalation of respiratory tract secretions. Likewise Berggren (3), employing the polarographic method of determining arterial pO_2 in bloods of subjects breathing 100 per cent O_2 , found that there was an average difference of 41 mm. pO_2 (between alveolar air and arterial blood) in 9 patients confined to bed as compared with 11 mm. in ambulatory subjects; the prolonged stay in the supine position had probably produced a mild degree of atelectasis.

Our experiments indicate that the O_2 consumption of whole heparinized human blood (exposed to a small gas bubble with an average pO_2 of 97.1 mm. at $38^\circ C$ for 10 min.) must be very slight. Krogh, working upon blood equilibrated for 1 minute came to a similar conclusion (16); he found that rapidly flowing blood had the same pO_2 as that trapped in a glass tonometer for 1 minute. Others have observed that the O_2 consumption of blood is very slight over short periods (17). In several instances, we equilibrated a second bubble of gas with the same arterial blood immediately after the first 10 minute equilibration and were unable to detect any significant difference in the two pO_2 determinations.

CONCLUSIONS

1. Using a direct method of measuring arterial O_2 tensions in 13 normal young adults at rest in the semi-recumbent position, an average figure of 97.1 mm. Hg was obtained.
2. Simultaneously collected alveolar air samples revealed an average pO_2 of 97.4 mm. Hg in end-expiratory samples.
3. There is no difference between alveolar and arterial pO_2 in healthy resting subjects at sea level.
4. The errors involved in previous measurements are discussed.

REFERENCES

- (1) BARCROFT, J. AND M. NAGAHASHI. *J. Physiol.* 55: 339, 1921.
- (2) BARCROFT, J., C. A. BINGER, A. V. BOCK, J. H. DOGGART, H. S. FORBES, G. HARROP, J. C. MEAKINS AND A. C. REDFIELD. *Philosoph. Trans. Roy. Soc. London* 211: 351, 1923.
- (3) BERGGREN, S. M. *Acta Physiol. Scand.* 4: Suppl. XI, 1942.
- (4) BERNTHAL, T. *Ann. Rev. Physiol.* 6: 171, 1944.
- (5) BEST, C. H. AND N. B. TAYLOR. *The physiological basis of medical practice.* 3rd ed., Williams & Wilkins Co., Baltimore, p. 530.

- (6) BOCK, A. V., D. B. DILL, H. T. EDWARDS, L. J. HENDERSON AND J. H. TALBOTT. *J. Physiol.* **68**: 277, 1929-30.
- (7) BOCK, A. V., H. FIELD, JR. AND G. S. ADAIR. *J. Biol. Chem.* **59**: 353, 1924.
- (8) BOOTHBY, W. M. Personal communication.
- (9) CULLEN, S. C. AND E. V. COOK. *This Journal* **137**: 238, 1942.
- (10) DOUGLAS, C. G. AND J. S. HALDANE. *J. Physiol.* **44**: 305, 1912.
- (11) DRABKIN, D. L. AND C. F. SCHMIDT. To be published.
- (12) HICK, F. K. *Proc. Soc. Exper. Biol. and Med.* **33**: 582, 1936.
- (13) KRAMER, K. *Ztschr. f. Biol.* **96**: 61, 1935.
- (14) KROGH, A. *Scand. Arch. Physiol.* **20**: 259, 1908.
- (15) KROGH, A. AND M. KROGH. *Scand. Arch. Physiol.* **23**: 179, 1910.
- (16) KROGH, A. *Scand. Arch. Physiol.* **23**: 193, 1910.
- (17) PETERING, H. G. AND F. DANIELS. *J. Am. Chem. Soc.* **60**: 2796, 1938.
- (18) RICHARDS, D. W., JR. In *Medical physics*, ed. by O. GLASSER. Yearbook Publishers, Chicago, 1944, p. 1228.
- (19) ROUGHTON, F. J. W., R. C. DARLING AND W. S. ROOT. *This Journal* **142**: 708, 1944.
- (20) SCHMIDT, C. F. In *MacLeod's Physiology in modern medicine*. 9th ed. C. V. Mosby Co., St. Louis, 1941, p. 622.
- (21) SCHOLANDER, P. F. *Rev. Sc. Instruments* **13**: 264, 1942.
- (22) VAN SLYKE, D. D. AND J. M. NEIL. *J. Biol. Chem.* **61**: 523, 1924.

FACTORS AFFECTING THE DETERMINATION OF OXYGEN CAPACITY, CONTENT AND PRESSURE IN HUMAN ARTERIAL BLOOD

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The oxygen capacity of blood is usually obtained by rotating a few cubic centimeters with air for about 15 minutes in a closed vessel of about 25 to 50 times the volume, and then analyzing the oxygen content of the equilibrated sample by the customary Van Slyke technique (1). Division of the oxygen capacity into the oxygen content of blood drawn direct from the artery (or vein), as also measured gasometrically, gives the percentage saturation of the hemoglobin of the blood with oxygen. The oxygen pressure (pO_2) corresponding to the measured percentage saturation (per cent O_2Hb) can then be read off from the dissociation curve of the blood, if this is known. Table 1 summarises the results on the per cent O_2Hb of normal human arterial blood at sea level as given by several recent groups of observers. The average figure for the per cent O_2Hb is close to 95 in each case, and the corresponding figure for the arterial pO_2 is about 80 mm. Hg. The latter value is about 20 mm. below the average value for the alveolar pO_2 at sea level, this being usually given as 100 mm.

When breathing low oxygen pressures, e.g., at altitudes of 10,000 feet or over, this difference between alveolar and arterial pO_2 tends to disappear, the two figures usually agreeing to within ± 4 mm. In such cases, as table 2 indicates, the determination of pO_2 from the dissociation curve and the per cent O_2Hb is much more accurate, since the steep rather than the flat part of the curve is in use in the calculation. The difference of oxygen pressure (ΔpO_2) between alveolar and arterial blood at sea level is, however, outside even the much larger experimental error met with on the flat part of the dissociation curve. There has consequently been much speculation as to the cause of the difference. Several possible factors have been advanced to explain why the arterial O_2Hb at sea level is 2.0 to 2.5 per cent lower than would be expected if complete equilibrium with the alveolar air had been obtained. (Table 2 shows that above 10,000 feet an error of 2 per cent O_2Hb could only cause an error of 2.5 mm. in pO_2 .) These factors may be listed as follows:

1. Incomplete pulmonary oxygenation of part of the blood owing either to unequal ventilation of different parts of the lung (Haldane, 5) or to by-passing of the venous blood via anastomoses or foramina Thebesii (Haldane, 5; Krogh, 6).

2. Oxygen consumption in the lungs or in the blood during the time taken to pass from the pulmonary vein to the systemic artery (Pflüger, 7).

3. Possibility that the pH of the red cells may be more acid at the moment when the blood leaves the lung capillaries than the final value after withdrawal from the systemic artery (Dill et al., 8).

4. The final stages of the chemical reaction of oxygen with hemoglobin in the red cell may be too slow for equilibrium to be reached during the time spent by the blood in the lung capillary (Roughton, 9; Bock et al., 10).

Whilst factor 1 is certainly important in abnormal conditions, there is no convincing evidence as to its significance in normal men. Factor 2 seems to have been eliminated by the control experiments of Barcroft et al. (11). As regards factor 3, physico-chemical theory indicates that if anything the pH of the red cells should be more alkaline in the lung capillaries than its final equilibrium value, since CO₂ must be lost relatively more rapidly from the red cells than from the plasma owing to the carbonic anhydrase and the hemoglobin-carbamino reaction only occurring in the former and not in the latter. The rapid reaction

TABLE 1
Values of arterial % O₂Hb at sea level in normal men

OBSERVERS	NUMBER OF CASES	SPREAD	MEAN
Harvard Fatigue Laboratory (2).....	154	93-98	95.8
Keys & Snell (3).....	87	93-98	95.0
Cullen & Cook (4).....	23	93.5-96.5	94.9
			95.2

TABLE 2
Error in calculation of arterial pO₂ corresponding to an error of 1% O₂Hb at various altitudes

ALTITUDE	AVERAGE % O ₂ Hb	ERROR IN pO ₂
<i>feet</i>		<i>mm.</i>
17,000	75	1.0
14,000	80	1.0
12,000	85	1.2
8,000	90	3.0
sea level	95	7.0
sea level	97	14.0

velocity experiments of Roughton et al. (12, 13) on the rate of passage of oxygen in and out of the red cells in vitro make it unlikely that factor 4 is important.

A further more general observation may also be made. On any of these explanations it should follow that the arterial pO₂ as determined directly by the aerotonometer bubble method should also be 20 mm. below the alveolar pO₂. The results of Barcroft and Nagahashi (14), however, show that such is not the case, the average Δ pO₂ given by their method (3 cases) being only 1 to 3 mm. Some quite other form of explanation of the discrepancy must therefore be sought.

The possibility occurred to us that instead of the O₂ content of the arterial blood, as drawn directly from the vessel, being 2 to 3 per cent too low, the O₂ capacity of the blood as customarily determined might be 2 to 3 per cent higher

than its value in the arterial circulating blood. Such a state of affairs would equally lead to a calculation of arterial per cent O_2Hb lower by 2 to 3 per cent than its true value in vivo. The first source of such an effect that we thought of arose from the work of Ammundsen (15) and others (16), which showed that the carbon monoxide combining capacity of human blood after treatment with $Na_2S_2O_4$ is about 3 per cent higher than the carbon monoxide combining capacity without such treatment. The difference is attributed to the presence of methemoglobin and/or other compounds containing iron in the ferric form. If such traces of methemoglobin, or similar compounds, slowly revert to ordinary hemoglobin after withdrawal from the body (such reversion is well known to occur quite fast in the case of blood containing appreciable methemoglobin; see 17, 18), then an in vitro augmentation of the oxygen capacity would occur. As a matter of fact in gasometric determinations of arterial per cent O_2Hb it is usual to analyze the O_2 content of the arterial sample immediately after withdrawal from the vessel, and only to determine the O_2 capacity at some indefinite time later.

The experiments to be described below not only confirm the results of Ammundsen (15) but also give fair evidence that about one-half of this initially inactive hemoglobin does revert to O_2 -combining hemoglobin on standing in vitro. Detailed analysis has, however, brought to light two other factors also tending to raise the O_2 capacity in vitro above its value in vivo. These factors are:

1. Rotation and drainage of blood in the saturating vessel is found to lead to a slight increase in concentration of the red cells, owing to the plasma sticking preferentially to the walls of the glass vessel and draining down less readily than the red cells.

2. In the case of blood containing appreciable amounts of CO, as in that of smokers, rotation with air may lead to a just significant replacement of COHb by O_2Hb especially if the temperature and illumination of the rotating vessel are high and if the ratio of the volume of air to that of the blood is great.

The demonstration of these effects has required certain modifications of the customary Van Slyke gasometric methods. These will be described first in the experimental section of the paper.

EXPERIMENTAL GASOMETRIC METHODS. Human heparinized blood was used in all the experiments.

Oxygen content of blood. This was determined by the usual gasometric procedure of Van Slyke (1).

Oxygen capacity of blood. 1. By 15 minutes' rotation of a few cubic centimeters blood in a closed vessel of volume 25 to 50 times greater; the vessel is then stood up, the cork removed, 1 cc. blood removed by pipette and its oxygen content measured in the constant volume Van Slyke apparatus in the usual way.

2. By a modification of the Sendroy method (19) of carrying out the whole capacity determinations in the Van Slyke apparatus. The reagents are the same as those used by Sendroy save that the saline solution is saturated with oxygen at one atmosphere and stored in a burette, and the ferrievanide-saponin

solution is deaerated in a tonometer and thence transferred anaerobically without foam to a burette where it is stored without any oil layer on top. The details of the procedure are as follows:

Two drops of caprylic alcohol and 1 cc. of O₂-saline are admitted into the Van Slyke chamber, and 1 cc. of the blood is then delivered into the Van Slyke cup, from which it is run into the chamber and washed in with 5 cc. of O₂-saline. The contents are mixed in the chamber, the excess of dissolved O₂ being sufficient to saturate the hemoglobin at once provided the latter was at least half-saturated at the start (this is always so with arterial samples: with very reduced venous samples 3 cc. extra of the saline could be used). The mixed solution is lowered till the mercury-blood meniscus is at the 50 cc. mark, the top tap being left open so that air at atmospheric pressure enters. After two minutes' shaking the air is quantitatively expelled and 0.24 cc. of the deaerated ferricyanide saponin added. The chamber is evacuated, the gases liberated and the CO₂ and O₂ absorbed in the manner described by Sendroy. To calculate the O₂ capacity, subtract from the O₂ pressure ($p_2 - p_1$) the c factor (usually 0.7 mm.) and the factor for the pressure of dissolved O₂ (= half the factor on the right hand scale of figure 49 of Peters and Van Slyke, Vol. II, p. 340).

Carbon monoxide content of blood. The procedure is mainly that of Horvath and Roughton (20) with certain modifications, the most important of which is the device described below for evading the "c" correction, the magnitude of which has been found to be somewhat variable. The other changes are in (a) the acid ferricyanide solution which now consists of a mixture of four parts of 32 per cent K₃Fe(CN)₆ with one part of acetate buffer made from 70 grams NaC₂H₃O₂, 3H₂O, 100 grams water and 15 cc. glacial acetic acid; (b) the substitution of 1.5 cc. of 4 per cent NaOH for 1.0 cc. of 10 per cent NaOH as absorbent of the liberated CO₂. The weaker NaOH solution causes less bubbling when added to the blood-ferricyanide mixture.

After the p_1 reading has been taken (= CO + a trace of N₂) a few cubic centimeters of 30 per cent NaCl are placed in the Van Slyke cup and the gas in the Van Slyke chamber compressed nearly to atmospheric pressure, so that only a small bubble remains at the top. The bore of the Van Slyke cup and the leads are cleared of mercury by running in a few drops of the NaCl solution, and either the whole or an aliquot portion of the gas bubble is forced up into the cup of an inverted Scholander-Roughton syringe (21), which has been previously filled with 30 per cent NaCl solution. For further details of this procedure see Appendix II of reference (21).

The length of the bubble before (= L_1) and after (= L_2) absorption with Winkler's solution is carefully measured in the capillary of the syringe, the residual trace of N₂ being very accurately obtained thereby.

The CO content then = $(p_1 - p_2) \times \frac{L_1 - L_2}{L_1} \times$ the usual temperature factor

where p_2 = the final Van Slyke reading after complete extrusion of all gas. With 2 cc. blood samples the experimental error is of the order of ± 0.01 vol. per cent

if the blood CO content is less than 2.0 vol. per cent. At higher contents the error increases roughly in proportion to the content.

Carbon monoxide capacity of blood with hydrosulfite (= total gasometric pigment of blood). The reagents required are the same as in the modified Horvath-Roughton method (v. supra), with the addition of a stock of distilled water saturated with CO at about one atmosphere and stored in a 100 cc. burette. The procedure is then as follows:

Five drops of caprylic alcohol are admitted in the Van Slyke chamber, and 1.5 cc. of 1 per cent saponin are placed in the cup. One cubic centimeter of the blood is introduced into the chamber in the usual way, followed by all the saponin. The two solutions are mixed in the chamber, and one minute is allowed for complete laking. Two cubic centimeters of hydrosulfite-borate are placed in the cup and 1.5 cc. admitted and mixed with the blood solution. After one minute for complete reduction of the O_2Hb 18 cc. of the CO-water are introduced, the top sealed and the solutions in the chamber completely mixed. The mercury is lowered to the 50 cc. mark, the chamber covered with black paper and shaken in the dark for two minutes. The extracted gas is quantitatively expelled and the process then repeated. Three cubic centimeters of the deaerated acid-ferri-cyanide solution are then placed in the cup, the lower 2 cc. admitted and the mixture evacuated and shaken five minutes. The CO_2 is then absorbed by means of 1.5 cc. deaerated 10 per cent NaOH, the p_1 reading taken, and the extracted gas analysed for residual N_2 as described under measurement of CO content.

The CO capacity then = $(p_1 - p_2) \times 1.02 \times \frac{L_1 - L_2}{L_1} \times$ the usual temperature

factor.

The figure 1.02 in this equation allows for the CO unextracted from the large volume of solution (i.e., 22 cc.) in the chamber.

Carbon monoxide capacity without hydrosulfite. The procedure is the same as above, save that the hydrosulfite is omitted from the borate solution. Alternatively the CO capacity may be obtained by adding the CO-content of the blood to the O_2 capacity (modified Sendroy).

EXPERIMENTAL RESULTS. *Constancy of total gasometric pigment.* Table 3 shows, in the case of four normal men, the course of the total CO capacity of the blood (with $Na_2S_2O_4$) after drawing and storage for various times in closed vessels. In the first three cases the value clearly remains constant, whilst in the fourth the value immediately after withdrawing is 0.22 vol. per cent below the mean obtained later, but this discrepancy is scarcely outside experimental error. It may be concluded that the total gasometric pigment is a constant characteristic of the blood for at least six hours, provided the blood is corked in a vessel which it fills almost completely.

On the other hand rotation of small amounts of blood in closed vessels of relatively large gas volume (25-50 times greater) leads, as stated in the introduction, to an increase in the total gasometric pigment. Since this finding is fundamental to the technique of other results to be given later, it will be convenient to describe at the outset the evidence on which it is based.

Effect of rotation in tonometer on gas-combining capacity of blood. The first experiments which led us to suspect this effect happened to be done at 37°C. with a gas volume to blood volume value of about 60 to 1.

It will be seen from table 4 that after the tonometer rotation the total gasometric pigment is increased on the average by 0.3 vol. per cent, i.e., by 1.5 per

TABLE 3
Course of total gasometric pigment with time

SUBJECT	TIME AFTER DRAWING	CO + HYDROSULPHITE
	<i>hrs.</i>	<i>vols. %</i>
J. W.	0	19.85
	2 $\frac{3}{4}$	19.90
	5 $\frac{1}{4}$	19.75
	6	19.74
W. R.	0	20.39
	2 $\frac{1}{2}$	20.31
	5 $\frac{1}{2}$	20.44
W. W.	0	21.78
	2	21.85
	4 $\frac{1}{4}$	21.92
	5 $\frac{1}{2}$	21.90
R. N.	0	19.75
	1 $\frac{1}{2}$	20.03
	3 $\frac{3}{4}$	20.00
	6 $\frac{1}{4}$	19.89

TABLE 4
Effect of rotation in tonometer at 37°C. on total gasometric pigment

SUBJECT	TOTAL CO CAPACITY IN VOLS. %		% INCREASE
	Before rotation	After rotation	
R. D.	18.55	18.75	1.1
E. T.	18.88	19.04	0.8
D. D.	17.99	18.26	1.5
H. B.	21.00	21.52	2.5
M. C.	19.87	20.04	0.8
A. B.	20.98	21.48	2.4
Average.....			1.5%

cent of its initial value. That there is a parallel increase in the O₂ capacity is shown by a similar set of experiments in which the O₂ capacity by the modified Sendroy method increased an average of 2.0 per cent. In these experiments allowance was made by inter- or extra-polation for slow rises in the O₂ capacity with time (see later).

We also have an equal amount of data with the tonometer rotation at room

temperature (25°C.), but here the effects were smaller, not on account of the lower temperature, but because much smaller gas/blood ratios happened to have been used. The importance of this last factor suggested that the cause of the observed effect lay in a separation of red cells and plasma during the drainage of the tonometer, the residual drainage films on the walls thereof containing relatively more plasma than red cells. The smaller the total blood volume used, the larger will be the proportion of "plasma-rich" dregs left on the tonometer wall, and therefore the greater the hemo-concentration in the blood which drains to the bottom of the tonometer and is drawn off for analysis.

The correctness of this view-point was established by experiments on the quantity and composition of the dregs. The procedure is as follows:

1. A tonometer of volume 280 cc. is weighed dry = W_1 grams.
2. Eight to 16 cc. blood are introduced, the tonometer corked and weighed again = W_2 grams. Volume of blood = $(W_2 - W_1)/\text{specific gravity of blood}$.
3. The tonometer is rotated 15 minutes, stood vertically and a blood sample removed for analysis as usual.

TABLE 5

Difference in composition of drained blood and "dregs" after saturation of blood in tonometer 280 cc. tonometer used throughout

VOL. BLOOD cc.	VOL. DREGS cc.	TOTAL CO CAPACITY (VOLS. %)			CORRECTION %	VOL. TONOM- ETER/VOL. BLOOD
		Blood	Dregs	Δ %		
15.90	0.25	18.68	15.75	16	0.25	17
13.80	0.26	20.89	16.89	19	0.36	20
14.00	0.40	22.97	19.62	14	0.40	20
7.70	0.29	21.56	17.32	19	0.70	36

4. As much as possible of the drained blood is sucked off, the tonometer corked and weighed again = W_3 grams. Volume of dregs = $(W_3 - W_1)/\text{specific gravity}$.

5. The dregs are completely leached out with five to six dosages of 3 cc. CO-saturated water. All these leachings are delivered into the Van Slyke apparatus and their total bound CO measured. Division of the latter by the volume of the dregs gives the CO capacity of the latter.

The results of four such experiments are shown in table 5. The fifth column in table 5, headed Δ , indicates the percentage by which the capacity of the dregs falls below that of the drained blood. The next column ("correction") gives the calculated amount by which the capacity of the drained blood exceeds that of the truly mixed blood. Thus in the case of the first sample

$$\begin{aligned} \text{The correction} &= 100 \times [1 - (15.90 \times 18.68 + 0.25 \times 15.75)/(15.90 + 0.25) (18.68)] \\ &= 0.25 \text{ per cent} \end{aligned}$$

The magnitude of the correction increases, as would be expected, as the ratio of tonometer volume to blood volume is increased. Thus with the latter at 36

to 1 the correction amounts to 0.70 per cent. Ordinarily in O₂ capacity determinations blood is rotated in a tonometer of 25 to 50 times its volume (1), and the O₂ capacity must therefore, on this account, be 0.5 to 1.0 per cent higher than the true value of the mixed blood; and at 60 to 1 would amount to 1.2 per cent, which may be compared with the figure of 1.5 per cent given in table 4.

Total nitrogen determinations on the drained blood and the dregs gave confirmatory results, though the effects observed were somewhat smaller.

To avoid the uncertainty caused by this drainage error, most of the O₂-capacity estimations given in the next section were done, not by the classical method of saturating the blood in a tonometer, but by the modified Sendroy method in the Van Slyke apparatus. That the two methods agree, when the drainage error is equalized, is shown by the results given in table 6. In this table, the second column represents the O₂ content of the blood, as collected in the ordinary way

TABLE 6

Comparison of classical O₂ capacity method (drainage error equalized) with modified Sendroy method

SUBJECT	O ₂ CAPACITY (VOLS. %)		% DIFFERENCE
	Classical method	Modified Sendroy method	
R. D.	17.82	17.64	-1.0
R. D.	17.65	18.25	+3.3
R. D.	18.86	18.47	-2.1
H. B.	21.28	20.88	-1.9
E. T.	18.32	18.02	-1.6
M. C.	18.74	18.95	+1.2
R. D.	17.98	18.06	+0.5
R. D.	17.77	18.03	+1.4
Average.....			0.0

from the tonometer after saturation and then transferred to the Van Slyke apparatus for direct O₂ estimation. In the third column, the same blood after transfer from the tonometer to the Van Slyke apparatus is submitted to the whole of the modified Sendroy procedure, instead of having its O₂ at once liberated by ferricyanide, as in the results of the second column. The average difference between the results given in the second and third columns is seen to be zero.

Difference between CO-capacity with Na₂S₂O₄ (total gasometric pigment) and CO-capacity without Na₂S₂O₄. In bovine blood, Taylor and Corell (16) find that the CO capacity with Na₂S₂O₄ averages about 5.0 per cent higher than the O₂ capacity, or about 4.0 per cent higher than the CO capacity without Na₂S₂O₄ assuming a blank CO content of 0.2 vol. per cent. In an extensive series of tests on 53 normal human bloods, Ammundsen (15) finds an average excess of 3.0 per cent. Her figures show a rather wide scatter (0-10 per cent) both for different individuals and for the same individual at different times. Our results are sum-

marized in table 7, and agree very satisfactorily with those of Ammundsen, both as regards the general average and as regards the results on particular individuals, which likewise show the marked variations at different times.

The scatter of our figures (0.7 per cent to 5.0 per cent) however, is less than half those of Ammundsen. This improvement is probably in part due to more accurate gasometric technique, and in part due to the determination of the CO capacity *without* $\text{Na}_2\text{S}_2\text{O}_4$ usually being done within a few minutes after withdrawal, thus reducing the uncertainty arising from the factor of time after withdrawal (this does not enter in the case of the CO capacity *with* $\text{Na}_2\text{S}_2\text{O}_4$). Most

TABLE 7

Excess of CO capacity with $\text{Na}_2\text{S}_2\text{O}_4$ over CO capacity without $\text{Na}_2\text{S}_2\text{O}_4$ expressed as percentage of latter

SUBJECT	OBSERVATIONS	MEAN
E. T.	4.1, 2.7, 3.5, 1.9, 3.3, 3.0	3.1
R. D.	3.3, 2.5, 1.9, 4.6, 1.7	2.8
H. B.	2.9, 2.4, 5.0, 0.7	2.8
M. C.	2.9, 4.6, 1.5	3.0
M. G.	4.1, 1.8	3.0
Others	1.8, 2.2, 1.4, 1.5, 4.8	2.3
General average of 26 tests.....		2.8%

TABLE 8

Changes in capacity of blood after drawing

SUBJECT	% INCREASE IN CAPACITY ON STANDING	MEAN
E. T.	2.2, 1.7, 0, 1.1	1.3
R. D.	0.3, 4.0, 3.7, 4.5, <u>4.0</u> , <u>1.7</u> , <u>0.7</u> , <u>2.0</u>	2.6
H. B.	1.2, 0.1, 2.3, <u>0.8</u>	1.1
M. G.	0.0, 1.1, <u>1.7</u>	0.9
Others	4.5, 2.2, 1.7, 1.5, 0.0, <u>1.1</u> , <u>0.8</u> , <u>0.3</u> , <u>-0.4</u> , <u>-1.4</u>	1.0
General average		1.5%
Average of later (underlined) results		1.1%

of the above results were obtained with venous blood, but in a few cases arterial blood was also used without any significant difference.

Changes in the O_2 -combining capacity of blood with time. Some 25 comparisons have been made of the O_2 capacity of normal human blood (modified Sendroy, method) within a few minutes after drawing from the vein or artery, with the O_2 capacity of the same blood after storage in closed vessels for varying periods up to 24 hours. The relevant data are given in table 8. In the majority of cases a slight increase, ranging from 1 per cent to 4.5 per cent, was observed within an hour after drawing, after which no detectable change occurred. In

ten cases the increase was positive but less than the experimental error (1 per cent), and in two cases a very small negative effect was observed. In one case a significant increase did not occur till after a latent period of 2 hours. Five parallel observations on the course of the CO capacity *without* Na₂S₂O₄ showed average effects of the same degree: in all but one of these the final plateau value was within experimental error, equal to the CO capacity *with* Na₂S₂O₄ which remained constant throughout. In these four particular cases the reversion of inactive hemoglobin to gas combining hemoglobin was apparently complete: this, however, is exceptional.

The underlined figures in table 8 are more reliable, since they represent the means of analyses by two independent observers and were obtained at a later and more accurate stage of the research. The average of the latter (1.1 per cent) is somewhat lower than that of the general average (1.5 per cent), but we believe it to be a safer measure of the true effect which is thus one-half or less of the average difference between CO capacity *with* and *without* Na₂S₂O₄. Although the magnitude of the effect is uncomfortably close to the limits of the experimental error, the fact that it is positive in all but two out of 30 tests makes us feel that it must be significant.

It would, of course, be far preferable to have a demonstration of the effect which does not depend on a small difference between two large quantities. If the change in question were a reversion from methemoglobin to hemoglobin, it would be very difficult to demonstrate optically if the quantities involved were only of the order of 1 to 2 per cent of the total pigment: if the inactive pigment resembles hemoglobin much more closely in optical properties than does methemoglobin, the problem would be still more difficult, if not impossible. A more promising line of approach would be to devise a continuous gasometric method of following the time-course of the gas combining capacity of a given sample of blood. Some preliminary experiments of this kind have already been done, and will be continued if other work permits.

Effect of traces of carboxyhemoglobin on oxygen-capacity of blood. The blood of smokers usually contains an average of about 1.0 vol. per cent of CO, which is equal to 5 per cent of the gas combining capacity of the blood. It was of interest to test whether the usual method of saturating the blood with air in a tonometer leads to an appreciable decrease in the CO bound to the hemoglobin, with an equivalent increase in the O₂ so bound. In three such cases it was found that there was a definite loss of CO amounting on the average to 0.08 vol. per cent per hour. In the usual 15 minutes this would indicate a gain of only 0.1 per cent in the oxygen combining capacity. This is only a second order effect but is in the same direction as the two larger effects previously found. At 38°C. the loss of CO was about four times greater than at 25°C. It would be expected that if the films of blood happened to be strongly illuminated during rotation, the loss of CO would be enhanced owing to the photochemical effect of light on the dissociation of COHb; this has not been directly verified.

DISCUSSION. Three factors have been shown to cause the oxygen capacity of blood, as determined by the usual method of saturation with air in a tonometer,

to be greater than its presumed value in vivo before withdrawal from artery or vein. Two of these factors, viz., the effect of drainage in the tonometer and the loss of CO during rotation with air, depend on the ratio of the volume of tonometer to the volume of blood, and have therefore been somewhat inconstant in previous work. Under average conditions they have probably amounted to about 0.8 per cent of the total capacity. The third factor, viz., reversion of some of the 3 per cent (average figure) inactive fraction of hemoglobin established to be present in normal human blood by Ammundsen (15) and ourselves, varies in an unpredictable manner, but on the average amounts to 1.1–1.5 per cent. The total sum of the three effects would be expected to be about 2 per cent, which would raise the average figures for the per cent O_2Hb in arterial blood from 95 to 97 at sea level. The latter figure is in close accord with the average value of 98 per cent O_2Hb , as reported recently by Drabkin and Schmidt (22). These authors' determinations were made by spectrophotometric technique on normal human arterial blood within minutes of withdrawal from the blood vessel. The pO_2 corresponding to 97 per cent O_2Hb is 98 mm., if read off from a standard dissociation curve: this value is very close to the average figure for the alveolar pO_2 of normal man at sea level. We believe that the previously claimed differences of 20 mm. Hg between alveolar air and arterial blood at sea level are in error, through inattention to these three factors. In view of the difficulty of allowing for these factors satisfactorily in given instances, it seems doubtful whether the continued use of the dissociation curve for determining arterial pO_2 at sea level is desirable: this caution does not, however, apply to experiments at altitude, where the percent O_2Hb is below 85, since in this range an uncertainty of 2 per cent O_2Hb only affects the determination of pO_2 to the extent of 2 to 3 mm. For sea level determinations of arterial pO_2 it would seem that wider use should be made of aerotonometer methods as developed by Barcroft and Nagahashi (14) and recently by Comroe (23) who, with new and improved technique, has obtained an average pO_2 in normal human arterial blood of 97.1 mm.

Another point of possible practical importance arises from the fact that colorimetric methods of estimating hemoglobin are usually standardized against the combining capacity as determined gasometrically. If an accuracy better than 2 to 3 per cent is desired attention should be paid to the effects recorded in this paper.

The 3 per cent excess of the CO capacity with $Na_2S_2O_4$ over the CO capacity without $Na_2S_2O_4$ may be compared with the 5 per cent excess found by Klumpp (24) and others, of the total Fe content of the blood over the Fe content of the active hemoglobin of the blood, as calculated from the CO capacity without $Na_2S_2O_4$. Only a very small part of the latter 5 per cent excess is due to plasma Fe: most of the remainder thus appears to consist of iron in ferric form (e.g., methemoglobin, hematin) which, after reduction with $Na_2S_2O_4$, is capable of combining with CO. That some of this remainder is probably methemoglobin is indicated not only by the results of table 8, but also by the known reversibility of the hemoglobin-methemoglobin redox system and its relationship to the naturally occurring reducing substances of the blood, e.g., glucose and lactate (see 17, 18 for references to previous work).

All the results reported in this paper are on normal human blood. It would be interesting to extend them to varied conditions, e.g., to polycythemia resulting from high altitude acclimatization to polycythemia vera, to anemia and to idiopathic cyanosis. In one case of the latter condition two years ago, which we wish to record here as there has been no previous opportunity of publishing the results we found the following curious figures:

1. By blood gasometric analysis—O₂ capacity 15.4 vols. per cent, CO content 0.4 vol. per cent, CO capacity without Na₂S₂O₄ 22.4 vols. per cent, CO capacity with Na₂S₂O₄ 25.3 vols. per cent.—Difference of two CO capacities = 2.9 vols. per cent.

2. By Evelyn photoelectric analysis—sulphemoglobin none, methemoglobin 2.5 vols. per cent, total hemoglobin as cyanmethemoglobin 19 vols. per cent.

3. Total pigment by conversion to alkaline hematin 22.8 vols. per cent.

The most remarkable features of this case are the large differences between the CO capacity and the O₂ capacity, and the indication of the presence of some compound capable of combining with CO but without showing the optical properties of hemoglobin or hematin. This type of case obviously calls for further investigation.

SUMMARY

1. Ammundsen's finding that addition of Na₂S₂O₄ to normal human blood raises the CO combining capacity by 3 per cent on the average is confirmed, thus indicating that 3 per cent of the pigment is inactive as regards gas combining power.

2. The oxygen capacity of arterial blood, as obtained by 15 minutes' saturation with air in a tonometer followed by analysis in the Van Slyke-Neil manometric apparatus, is considered to be about 2 per cent higher than the value in the blood at the moment of withdrawal from the vessel. Three factors contribute to this discrepancy:

a. Drainage errors in the saturator: these cause the blood sample removed for analysis to be unduly rich in red cells (average effect equals about 0.7 per cent).

b. Gradual reversion of some of the inactive pigment to the active gas combining form. This effect is very variable, but on the average equals 1.0 to 1.5 per cent of the total capacity. Owing to the smallness of the effect, the nature of the inactive, reverting pigment has not been established. It may be in part methemoglobin.

c. Presence of traces of COHb in the blood.

3. Correction for these factors raises the average per cent O₂Hb of normal men at sea level from 95 to 97: the arterial pO₂, as calculated from these figures and the blood dissociation curves, comes out to 100 mm., in agreement with the alveolar pO₂, rather than to the average figure of 80 mm. given in recent papers. The latter figure is believed to be false.

4. The calculation of arterial pO₂ from arterial per cent O₂Hb and the dissociation curve should only be used when the per cent O₂Hb does not exceed 85, e.g., at altitudes above 10,000 feet: at lower altitudes the arterial pO₂ should be determined aerotonometrically.

REFERENCES

- (1) PETERS, J. P. AND D. D. VAN SLYKE. Quantitative clinical chemistry. Baltimore, Vol. 2, 1932.
- (2) Harvard Fatigue Laboratory. Unpublished observations.
- (3) KEYS, A. AND A. SNELL. *J. Clin. Investigation* 17: 59, 1938.
- (4) CULLEN, S. C. AND E. V. COOK. *This Journal* 137: 238, 1942.
- (5) HALDANE, J. S. *Respiration*. New Haven, 1935.
- (6) KROGH, A. *The comparative physiology of respiratory mechanisms*. Philadelphia, 1941.
- (7) PFLÜGER. Quoted by J. S. HALDANE AND J. LORRAIN SMITH. *J. Physiol.* 22: 231, 1897.
- (8) DILL, D. B., E. H. CHRISTENSEN AND H. T. EDWARDS. *This Journal* 115: 530, 1936.
- (9) ROUGHTON, F. J. W. Ph.D. Thesis, Cambridge University, 1925.
- (10) BOCK, A. V., D. B. DILL, H. T. EDWARDS, L. J. HENDERSON AND J. H. TALBOTT. *J. Physiol.* 68: 277, 1929.
- (11) BARCROFT, J., A. COOKE, H. HARTRIDGE, T. R. PARSONS AND W. PARSONS. *J. Physiol.* 53: 450, 1920.
- (12) HARTRIDGE, H. AND F. J. W. ROUGHTON. *J. Physiol.* 62: 232, 1927.
- (13) BATEMAN, J. B. AND F. J. W. ROUGHTON. *Biochem. J.* 29: 2630, 1936.
- (14) BARCROFT, J. AND M. NAGAHASHI. *J. Physiol.* 55: 339, 1921.
- (15) AMMUNDSEN, E. *J. Biol. Chem.* 138: 563, 1941.
- (16) TAYLOR, D. S. AND C. O. CORYELL. *J. Am. Chem. Soc.* 60: 1177, 1938.
- (17) WENDEL, W. B. *J. Clin. Investigation* 18: 179, 1939.
- (18) DARLING, R. C. AND F. J. W. ROUGHTON. *This Journal* 137: 56, 1942.
- (19) SENDROY, J. *J. Biol. Chem.* 91: 307, 1931.
- (20) HORVATH, S. AND F. J. W. ROUGHTON. *J. Biol. Chem.* 144: 747, 1942.
- (21) SCHOLANDER, P. AND F. J. W. ROUGHTON. *J. Biol. Chem.* 148: 551, 1943.
- (22) DRABKIN, D. L. AND C. F. SCHMIDT. *Am. J. Med. Sci.* 208: 133, 135, 1944, and "To be published."
- (23) COMROE, J. H., JR. *Am. J. Med. Sci.* 208: 135, 1944, and "To be published."
- (24) KLUMPP, T. G. *J. Clin. Investigation* 14: 351, 1935.

INFLUENCE OF DYING GASPS, YAWNS AND SIGHS ON BLOOD PRESSURE AND BLOOD FLOW¹

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Earlier studies have demonstrated that interesting and important circulatory changes accompany excessively prolonged or severe expiratory activities such as crying, coughing and straining (1, 2, 3, 4, 5). Circulatory changes would also be expected to accompany excessively prolonged or severe inspiratory acts such as dying gasps, yawns and sighs. Yet, none are described in the current literature and textbooks (6, 7, 8, 9). It is interesting that Stephen Hales in 1733 (10) observed that deep sighing and respiratory efforts of dying mares increased systematic arterial pressure. He believed the greater motion of the lungs caused the blood to pass more freely and in greater quantity to the left heart.

The observations reported in the present study are limited principally to the effects of normal respiration, deep breathing and dying gasps, since yawns and sighs could not be induced experimentally in dogs. However, the data have allowed appreciation of the hemodynamic changes induced by sighs and yawns.

METHODS. Morphine sulfate, 5 mgm. per kgm., was administered subcutaneously 30 minutes prior to the experiments. All operative procedures were accomplished with the aid of local infiltrations of 1 per cent procaine hydrochloride. In those experiments where it was necessary to enter the chest, the appropriate intercostal nerves were blocked.

Changes in the pressure relationships between the thoracic and abdominal cavities were measured since they modify venous return from the abdominal reservoirs to the right heart. Balloons fastened to leaden tubes and inserted into the abdominal and thoracic cavities were connected to a differential manometer (4) so that the intrathoracic pressure was subtracted from the abdominal pressure. This differential pressure has been called the abdominal thoracic pressure gradient. Changes in this gradient modify venous return to the right heart and can result from unequal increases or decreases in the intrathoracic or intra-abdominal pressures. When it was unnecessary to enter the chest, the balloons were inserted through the mouth and into the esophagus or stomach. Long balloons only partially filled were used since they tend to dissipate pressure changes from the activity of the alimentary canal (11) but register the changes transmitted to them from the intrapleural space and abdominal cavity. These

¹ The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Georgia School of Medicine.

² Now at Division of Pharmacology, University of California Medical School, San Francisco.

esophageal and gastric pressures were considered to change in parallel with thoracic and abdominal pressures.

Hollow sounds constructed from stainless steel tubing G 12 to 16 and adapted to the size of the animal were connected to other optical manometers and were

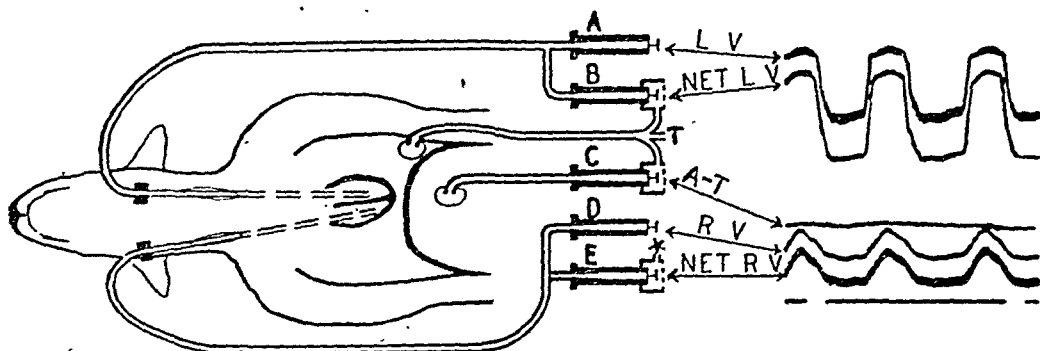


Fig. 1. Diagram showing method of recording gross pressures and net pressures in unanesthetized animals with closed chests. Hollow stainless steel sounds were inserted in the left and right ventricles and balloons were placed in the thoracic and abdominal cavities.

The net pressure is the actual effective pressure and is the gross pressure in the structure or organ minus the pressure acting on the outside of that structure or organ (1).

Manometer A records the left ventricular pressure (L.V.).

Manometer B records the net left ventricular pressure (Net L.V.) which is the ventricular pressure minus the thoracic pressure acting on the outside of the ventricle. Increases in the thoracic pressure may increase the left ventricular pressure but it also compresses the coronary vessels. Therefore the thoracic pressure must be subtracted from the systemic arterial pressure in order to evaluate the *effective* net pressure to the coronaries. This was accomplished by means of the differential manometer B where the left ventricular pressure is led to the back of the manometer and the thoracic pressure is led to the front chamber of the manometer.

Manometer D records the right ventricular pressure (R.V.).

Manometer E records the net right ventricular pressure (Net R.V.). This is the right ventricular pressure minus the thoracic pressure acting on the outside of the ventricle. Increases in the thoracic pressure may increase the right ventricular pressure but this is a mere passive rise since it also increases the pressure on the outside of the pulmonary vessels. Therefore the thoracic pressure must be subtracted from the pulmonary arterial pressure in order to evaluate the *effective* net pressures in the pulmonary circulation. This was accomplished by means of the differential manometer E in a manner similar to that described above for manometer B.

Manometer C records the abdominal thoracic pressure gradient (A-T). This is the abdominal pressure minus the thoracic pressure and is the *effective* net pressure which facilitates or hinders blood flow from the abdominal venous reservoir (inferior vena cava and portal system) to the right heart. Increases in the abdominal pressures effectively increase blood flow to the right heart only when a corresponding rise in thoracic pressure does not occur.

The tube "T" from the balloon in the thorax is also connected to the front chamber of the manometer E, see *.

inserted 1, down the left carotid and into the left ventricle, and 2, down the right external jugular and into the right ventricle. Saline infused through these sounds at the rate of about 1 cc. per minute reduced the danger of obstruction by coagulation. This made it possible to obtain continuous simultaneous pressure

records from the left and right ventricles in the closed chest. Both *gross* and *net* ventricular pressures were obtained. The *gross* pressures were obtained with simple manometers (1), as commonly measured from the ventricular cavities and show both active and passive changes. The *net* ventricular pressures are the gross pressures minus the intrathoracic pressure and were obtained with differential manometers (4) where the ventricular pressures were led to the rear chambers and the intrathoracic pressure was led to the front chambers of the manometers in the usual manner, see figure 1. These net ventricular pressures show only active changes and are the pressures actually distending the heart cavities. They are the effective blood pressures to the blood vessels and organs within the thorax. The net left ventricular systolic pressure can be considered equivalent to the net coronary systolic pressure. The net right ventricular systolic pressure can be considered equivalent to the net pulmonary arterial systolic pressure. In addition to the net coronary systolic and the net pulmonary arterial systolic pressures, when necessary to determine net pulmonary venous and net inferior vena cava pressures, they can be obtained from such records by considering the following as equivalents: the net right ventricular diastolic pressure is equivalent to the net inferior vena cava pressure; the net left ventricular diastolic pressure is equivalent to the net pulmonary venous pressure.

Similar methods (1), supplied measurements of the *gross* and *net* systemic arterial pressure to the central nervous system. The gross systemic arterial pressure acts to push blood to organs including the brain and spinal cord. The pressure within the craniospinal cavity acts to hinder blood inflow. Therefore, the effective arterial pressure to the central nervous system is the arterial pressure minus the cerebrospinal fluid pressure. This effective pressure has been called the net arterial pressure to the brain and spinal cord and has been calculated from simultaneous measurements of the arterial pressures and cerebrospinal fluid pressures. The cerebrospinal fluid pressure was recorded from no. 18 or no. 20 G needles inserted in three animals into the subarachnoid space in the region of the first or second lumbar vertebra.

Acute cardiac arrest was produced by electrically induced ventricular fibrillation. In four dogs the electrodes were placed upon the ventricles while inserting the thoracic balloons. The chest was then closed and the pneumothorax was reduced. In two other animals where the chest was not opened an insulated sound with the end serving as the stimulating electrode was introduced down the external jugular into the right ventricle. An indifferent electrode was placed upon the skin over the heart. The various gross and net pressures were then recorded while stimuli were applied to the ventricle using two batteries and a Harvard Inductorium at full strength.

RESULTS AND DISCUSSION. First the effects of normal respiration will be described. As shown in figure 2A, normal inspiration (arrow) lowers the thoracic pressure and increases the abdominal thoracic pressure gradient which increases venous return to the heart from the portal system and the inferior vena cava. During inspiration the net right ventricular pressure pulses show a higher pulse pressure and a peak with a wider plateau, see second and third NR pressure pulses of figure 2A. These changes indicate a larger and more prolonged effec-

tive ejection period without any appreciable change in the duration of systole. These changes are interpreted as characteristic of an increased right cardiac output secondary to an increased filling with inspiration.

When ventricular fibrillation was induced with strong tetanizing electrical stimuli applied directly to the heart in the closed chest, respiratory activity continued for several minutes after effective cardiac action had ceased. The deep forceful natural breathing (see fig. 2B) produced significant changes in the blood pressure and circulated blood. During inspirations (arrows) the abdominal thoracic pressure gradient is increased; the *gross* right ventricular pressures returned toward zero; but the effective *net* right ventricular pressure (NR) increased. As shown in figure 2B, it reached values between 25 and 30 mm. Hg

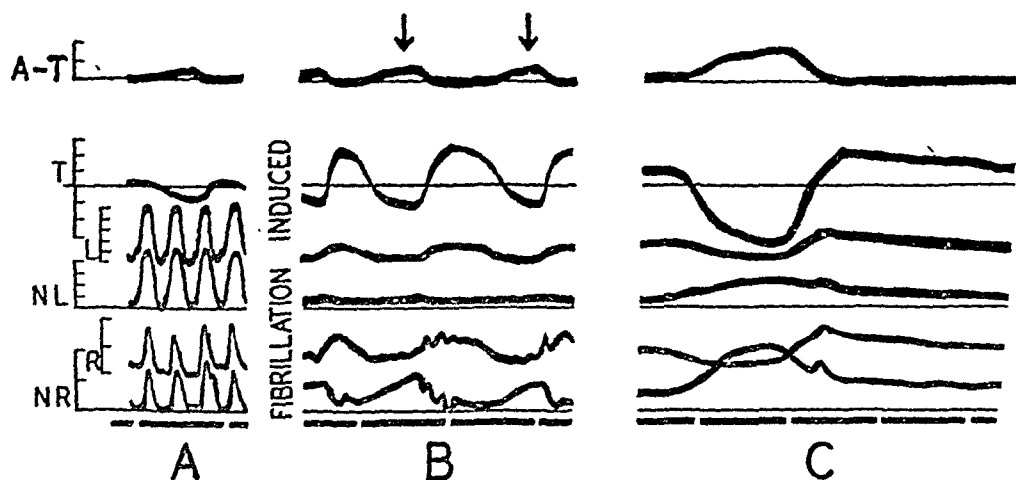


Fig. 2. Reproduction of pressure pulses from dog, from above downwards: abdominal thoracic pressure gradient (A.-T.), thoracic (T), left ventricular (L), net left ventricular (N.L.), right ventricular (R) and net right ventricular (N.R.). Time line is interrupted at two second intervals. Pressure scales are shown in units of 25 mm. Hg in all except that of the thoracic cavity where units are + 10 and - 10 from zero.

A. Normal tracings.

B. Tracings obtained one minute after induction of ventricular fibrillation and when the heart is not pumping any blood.

C. Tracings obtained one minute later during a dying gasp.

which were approximately equal to those produced by the right ventricle when it was contracting (fig. 2A). Simultaneously these deep respiratory efforts produced only small changes in the effective *net* left ventricular pressures (NL). It can be concluded from these data that repeated deep forceful breathing, even in the presence of complete cardiac arrest, repeatedly increased venous return to the right ventricle and to the pulmonary vessels while moving a smaller quantity of blood from the pulmonary vessels on into the left ventricle. This also means that deep forceful breathing in the presence of cardiac failure can contribute to pulmonary engorgement by transferring blood from the abdominal venous reservoirs to the pulmonary vessels.

Since sighs and yawns are modified deep breaths they also should increase venous return to the right heart. It has been observed by one of the authors that small children when asleep will usually yawn if they are lifted into an upright

position. Sometimes the yawn would occur repeatedly each time they were changed to an upright position. This yawn could be physiologically required to increase air exchange or to increase venous return. There is no reason to expect any real need of increased air exchange. However, it is conceivable that a sudden change to an upright position in an individual whose muscles are relaxed would retard venous return sufficiently so that a mechanism to increase venous return operates.

A yawn to be truly satisfying frequently is accompanied by a stretch. When present the stretch not only contributes to venous return but also, like the dying gasp, tends to divert blood to the heart and central nervous system.

Circulatory changes are pronounced during dying gasps. The effective *net* right ventricular pressure is elevated to values as high as 50 mm. Hg (see fig. 2C). The pulmonary vessels become so engorged that blood is pushed through to the left ventricle and elevates the effective *net* pressure in that chamber to 30 to 50 mm. Hg.

These dying gasps not only increase venous return but they cause significant coronary blood flow. Blood is pushed or diverted into the coronary vessels as a result of the extensive skeletal muscle activity and the elevated abdominal pressure, both of which squeeze blood vessels and increase peripheral resistance. Simultaneously the intrathoracic pressure is reduced so that peripheral resistance in the coronary vessels is lowered while the effective *net* coronary arterial pressure is elevated.

In similar experiments *net* cerebrospinal arterial pressures of 20 to 40 mm. Hg were produced by dying gasps. Therefore blood flow to the central nervous system is also accomplished by dying gasps. The mechanisms responsible for blood flow to the central nervous system were only slightly less effective than those described above which produced coronary flow, since dying gasps lower the cerebrospinal fluid pressure only slightly less than they lower the intrathoracic pressure. This is due to the fact that the cerebrospinal fluid pressure is influenced by the algebraic sum of the intrathoracic and abdominal pressure, though unpublished data indicate that it follows the intrathoracic pressure more closely than it follows the abdominal pressure (12).

These gasps produce air exchange and move blood to the vital areas, whereas mechanical resuscitators provide air exchange but do not cause any significant blood flow to the vital areas (13).

Cases have been encountered (12, 14) where patients apparently dead and without any evidence of heart action have again developed palpable pulse and audible heart contractions immediately after making dying gasps. Similar observations have probably been passed off with the remark "I must have been wrong when I failed to observe any pulse the first time." It is conceivable however that the blood pumping action of the dying gasps contributed to the return of effective cardiac contractions.

SUMMARY AND CONCLUSIONS

Gross and *net*, left and right ventricular pressures are recorded from dogs without operative entrance into the chest by means of hollow sounds inserted down

the left carotid into the left ventricle and down the right jugular into the right ventricle.

Normal inspiration increases venous return to the right heart and produces contour changes characteristic of larger and more prolonged effective ejection without significantly changing the duration of systole.

Dying gasps, deep breathing, yawns and sighs which are generally considered as respiratory acts, markedly increase venous return. In the presence of cardiac arrest, dying gasps pump blood through the lungs and temporarily provide blood flow to the vital areas, the central nervous system and heart. Effective *net* pressure as great as 50 mm. Hg in the pulmonary artery, 50 mm. Hg in the coronary arteries and 40 mm. Hg in the central nervous system arteries were created by dying gasps in dogs where cardiac action had ceased.

REFERENCES

- (1) HAMILTON, W. F., R. A. WOODBURY AND H. T. HARPER, JR. *J. A. M. A.* 107: 853, 1936.
- (2) WOODBURY, R. A., M. ROBINOW AND W. F. HAMILTON. *This Journal* 122: 472, 1938.
- (3) WOODBURY, R. A., E. E. MURPHEY AND W. F. HAMILTON. *Arch. Int. Med.* 65: 752, 1940.
- (4) WOODBURY, R. A., W. F. HAMILTON AND R. TORPIN. *This Journal* 121: 640, 1938.
- (5) HAMILTON, W. F., R. A. WOODBURY AND H. T. HARPER, JR. *This Journal* 141: 42, 1944.
- (6) HOWELL, W. H. *Textbook of physiology*. Ed. 13, p. 756. W. B. Saunders Co., Philadelphia, 1936.
- (7) WIGGERS, C. J. *Physiology in health and disease*. Ed. 3, p. 386, Lea and Febiger, Philadelphia, 1939.
- (8) EVANS, C. L. *Starling's Principles of human physiology*. Ed. 8, p. 856, Lea and Febiger, Philadelphia, 1941.
- (9) CARLSON, A. J. AND V. JOHNSON. *Machinery of the body*, p. 241, University of Chicago Press, Chicago, 1937.
- (10) HALES, STEPHEN. *Statistical Essays*, Vol. 2, London, 1733.
- (11) BRODY, D. A., J. M. WERLE, J. MESCHAN AND J. P. QUIGLEY. *This Journal* 130: 791, 1940.
- (12) Personal observation.
- (13) VOLPITTO, P. P., R. A. WOODBURY AND B. E. ABREU. *J. A. M. A.* (in press).
- (14) MEANS, J. H. Personal communication.

HEMOGLOBIN CONCENTRATIONS, RED CELL COUNTS AND ERYTHROCYTE VOLUMES OF COLLEGE WOMEN OF THE NORTH CENTRAL STATES¹

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Numerous hematological studies have been made of normal young women (1, 2, 3, 4, 5).

Osgood (3) summarized available data from the literature and reported the mean hemoglobin concentration of 369 women from the United States and Northern Europe to be 13.9 grams per 100 cc. blood. Average of erythrocyte counts for the same series was 4.85 million cells per cmm. and cell volume 41.8 per cent. Recent studies (1, 4, 6) have reported concentrations of hemoglobin ranging from 10.0 to 14.7 grams per 100 cc. blood of young women of the North Central States as against 12.0 to 16.0 grams recorded earlier as a result of work done on either coast (3, 7), or at the altitude of Denver (8). Red blood counts and erythrocyte volumes were found to be correspondingly lower in the North Central States. The possibility of a generalized low hemoglobin concentration existing among women living in the Plains states cannot be ignored and, if it exists, requires interpretation, particularly with respect to the assessment of nutritional status, since a hemoglobin lower than existing standards quite generally is accepted by the medical profession as a measure of a reduced physical reserve. However, refinements of technique and differences in sampling methods also could explain recorded results and, before sweeping conclusions can be drawn, more adequate measures of populations are desirable, particularly where hematological studies can be correlated with indices of nutritional status.

The workers in the North Central States studying the nutritional status of college women have determined the hemoglobin concentrations, erythrocyte counts and cell volumes of the bloods of a large sample of college women. This paper presents hematological data on 4,550 women. Material is included from the nutritional laboratories of Iowa State College, Kansas State College, Uni-

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² With the assistance of Virginia Minnich, Katherine Davis Widness, Peggy Rehm (deceased, February 1941), Anne Barman Caldwell, Agnes Leith Nordin. Journal Paper no. J-1206 of the Iowa Agricultural Experiment Station, Ames. Project 538.

³ Contribution no. 122, Department of Home Economics, Kansas Agricultural Experiment Station.

⁴ Paper no. 2167, Scientific Journal series, University of Minnesota.

⁵ Approved for Publication by the Director of the Nebraska Agricultural Experiment Station as paper no. 258, Journal Series.

⁶ Approved for publication by the Oklahoma Agricultural Experiment Station.

⁷ With the assistance of Jane Meiller and Lola Amidon Hill. Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

versity of Minnesota, University of Nebraska, Oklahoma Agricultural and Mechanical College and University of Wisconsin.

MATERIALS AND METHODS. The data reported herein were accumulated from September, 1935, through May, 1943. College women from 16 to 30 years of age were subjects for the investigation. More than 90 per cent of the group were from 17 to 24 years of age. Students were drawn from all college classes, many of them serving as experimental subjects for two to four years. Students on whom tests were made a second and third year have been considered as new individuals for each school year of study. At Iowa State College studies of hemoglobin concentration only were made on each entering class of women from 1937 through 1941, inclusive, and at the University of Nebraska, hemoglobin concentration only was studied.

One of the objectives of the work was to measure the incidence and distribution of low hemoglobin concentrations among essentially normal young women. Although certain measures of physical status were obtained on a large proportion of the subjects studied, no effort was made to screen the sample on which this report is based. The fact that each student was carrying an active college program precluded the presence of severe debilitating disease, and macrocytic anemias are rarely found in this age group (9).

Comparable techniques for determination of the blood values were used by the co-operating laboratories, and measuring instruments for all tests were calibrated by the United States Bureau of Standards. Table 1 records the methods used for the determination of hemoglobin. For red cell counts, two trenner pipettes were used and two counting chambers of the improved Neubauer counting instrument were filled from each pipette and 80 small squares counted from each chamber, a total of four counts for each blood sample. If, after inspection for equality of distribution of cells, a chamber was accepted for counting, the result was recorded and the average of four counts was taken as the number of erythrocytes per cmm. of blood for the individual, regardless of agreement between chambers. Magath, Berkson and Hurn (10) have shown the improbability of duplicating successive counts on the individual and that an attempt to select counts agreeing within any fixed range violates the principle of random sampling on which the test is based. Cell volume was determined by means of the Van Allen tube (11). Duplicate tests were made using heparin as the anti-coagulant (12).

RESULTS AND DISCUSSION. The distribution of all tests of hemoglobin concentration, red cell count and cell volume is recorded in figures 1, 2 and 3. Table 2 gives the data for each school with the mean, standard deviation and standard error for each series of tests. Preliminary examination of the records indicated that there were no age differences in the results.

The series tested at each school are presented separately since some variation between schools was found. Mean hemoglobin concentrations per 100 cc. of blood by states were as follows: Iowa 13.4, Kansas 13.4, Minnesota 13.0, Nebraska 13.3, Oklahoma 13.5 and Wisconsin 13.7 grams. In general, the erythrocyte counts and the cell volumes followed the variations in the hemo-

globin. Minnesota women with a low mean hemoglobin concentration of 13.0 grams per 100 cc. blood have an average red count of only 4.23 million cells per cmm., while the highest average hemoglobin concentration recorded (Wisconsin 13.7 grams) was associated with the highest red cell count, 4.75 million. Mean hemoglobin for the entire series was 13.4 grams per 100 cc. blood, erythrocytes 4.56 million cells per cmm. and cell volume 40.9 per cent. Estimations of variability of both the individual samples and the means are comparable since stand-

TABLE 1

Experimental methods and plans used for determination of the concentration of hemoglobin

STATE	CONDITIONS UNDER WHICH SAMPLE WAS TAKEN	SOURCE OF BLOOD	DETAIL OF HEMOGLOBIN TEST	
			Chemical method	Reading instrument
Iowa	Blood drawn between 8 a.m. and noon. Light breakfast	Capillary	Acid hematin (13)	Duboscq colorimeter* Electro-scopometer
Kansas	As above	Capillary	Acid hematin (13)	Duboscq colorimeter*
Minnesota	Blood drawn under basal conditions	Capillary	Acid hematin (13)	Duboscq colorimeter*
Nebraska	Blood drawn between 8 a.m. and noon. Light breakfast	Venous	Oxyhemoglobin (14)	Sheard Sanford photometer
Oklahoma	Blood drawn between 8 a.m. and noon. Light breakfast	Capillary	Acid hematin (13)	Duboscq colorimeter*
Wisconsin		Capillary	Acid hematin (13)	Duboscq colorimeter*

* Equipped with permanent Newcomber glass standard and blue filter. All reading instruments were standardized by oxygen capacity studies (15).

ard deviations ranged from 0.89 to 1.24 grams hemoglobin per 100 cc. blood and standard errors from 0.022 to 0.074. Measures of the variability of the red counts and the tests for cell volume were of similar magnitude. It is noteworthy that the lowest mean concentration of hemoglobin and also the lowest standard deviation were found at the University of Minnesota where basal conditions were maintained.

Seventy-two per cent of all hemoglobin tests fell within the mean plus or minus one standard deviation or between 12.2 and 14.6 grams per 100 cc. blood. Like-

wise, 71 per cent of all erythrocyte counts and 70 per cent of all cell volume tests were found within a range limited by the respective means plus or minus one standard deviation. Thus more than two-thirds of the red cell counts were between 4.18 and 4.95 million cells per cmm. whereas the comparable range of cell volume tests was from 38.3 to 43.5 per cent.

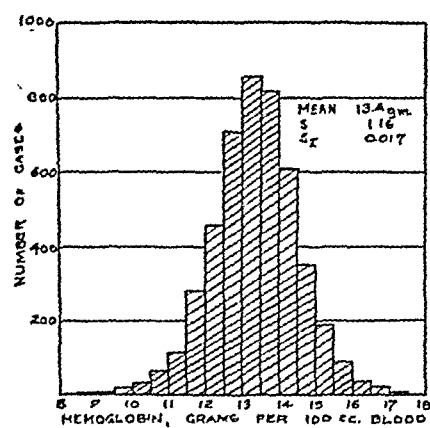


Fig. 1

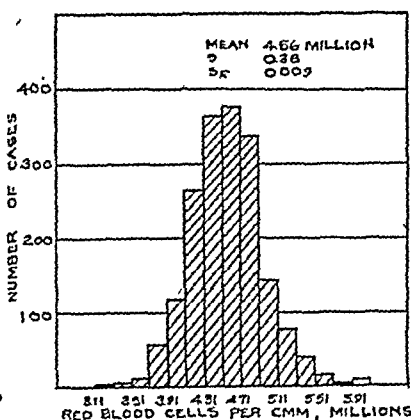


Fig. 2

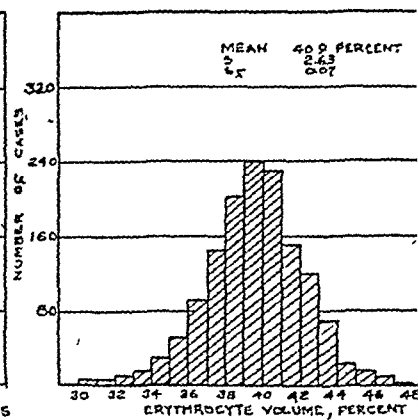


Fig. 3

Fig. 1. Distribution of 4550 hemoglobin tests.

Fig. 2. Distribution of 1792 red cell counts.

Fig. 3. Distribution of 1398 tests of erythrocyte volume.

TABLE 2

Hemoglobin concentrations, red cell counts and cell volumes of college women of the north central states

STATE	HEMOGLOBIN				RED CELL COUNT				CELL VOLUME			
	No. cases	Mean	s	s _x	No. cases	Mean	s	s _x	No. cases	Mean	s	s _x
Iowa.....	2934	13.4	1.18	0.022	804	4.64	0.39	0.014	602	41.4	2.28	0.093
Kansas.....	340	13.4	1.16	0.063	349	4.66	0.28	0.015	224	42.2	2.17	0.145
Minnesota..	292	13.0	0.89	0.052	292	4.23	0.31	0.018	292	39.0	2.25	0.132
Nebraska...	445	13.3	1.04	0.045								
Oklahoma..	284	13.3	1.24	0.074	284	4.54	0.34	0.020	280	40.2	2.90	0.173
Wisconsin..	225	13.7	1.11	0.066	63	4.75	0.42	0.053				
	4550	13.4	1.16	0.017	1792	4.56	0.38	0.009	1398	40.9	2.63	0.070

Maximum ranges of variation for each of the three tests reported in this paper are: hemoglobin, 8.5 to 17.5 grams per 100 cc. blood, erythrocyte count 3.11 to 5.91 million cells per cmm., and cell volume 32.0 to 48.1 per cent. The series here reported is much larger than any previously recorded and the ranges found for erythrocytes and total cell volume are no greater than those for hemoglobin since, in each case, the mean plus or minus two standard deviations measures a range 17, 13 and 17 per cent of the respective means. That the measures should yield similar degrees of variation is to be expected since the physiological relationship of the tests is obvious. However, these figures suggest that if only one

measure of the normalcy of the red blood cell system is to be obtained, the erythrocyte count may be subject to less sampling variation than either of the other two tests.

In all schools variations in the yearly samples from each state were at least as wide as the variations between states. Mean hemoglobin concentrations for a seven year period are recorded in table 3. In each case red cell counts and

TABLE 3

Yearly variations in hemoglobin concentration of college women from six states

YEAR OF STUDY	STATE					
	Iowa	Kansas	Minnesota	Nebraska	Oklahoma	Wisconsin
	No. Hb., gm. per 100 cc.	No. Hb., gm. per 100 cc.	No. Hb., gm. per 100 cc.	No. Hb., gm. per 100 cc.	No. Hb., gm. per 100 cc.	No. Hb., gm. per 100 cc.
1936-37	177 12.5 \pm 1.14		41 13.1			99 14.2 \pm 1.02
1937-38	525 13.0 \pm 0.86	88 13.2 \pm 1.25	37 13.4 \pm 0.69	12 12.3		69 13.7 \pm 1.13
1938-39	365 13.9 \pm 0.85	54 14.0 \pm 1.24	19 13.0	11 13.8	48 13.0 \pm 1.50	28 13.5 \pm 0.78
1939-40	524 12.5 \pm 0.08			19 13.2	11 13.9	
1940-41	574 14.0 \pm 1.17			116† 13.2 \pm 1.11		
1941-42	529 13.9 \pm 1.04			20 13.2		
1942-43				63‡ 13.3 \pm 0.94		
				39 12.8 \pm 1.12		
				50 13.3 \pm 0.89		
				101‡ 13.0 \pm 0.85		
Entire series	2694 13.4 \pm 1.18	142 13.5 \pm 1.30	97 13.1 \pm 0.89	431 13.3 \pm 1.04	59 13.2 \pm 1.15	196 13.9 \pm 1.14

* The date in each case represents the school year which extended from September through May. Except in the case of Nebraska and Wisconsin, the first observation on the entering student was used, in the two latter states, the first contact with the student is that reported but the student was not necessarily a freshman.

† Students from Union College, Lincoln, Nebraska.

‡ Students from Nebraska Wesleyan College.

erythrocyte volumes varied in the same direction but are omitted for economy of space.

SUMMARY AND CONCLUSIONS

Studies of hemoglobin concentrations, erythrocyte counts and cell volume tests are presented on young women from six colleges and universities located in the North Central States. The mean hemoglobin concentration was 13.4 grams per 100 cc. blood with a standard error of 0.017. Mean erythrocyte count was 4.56 ± 0.009 million cells per cmm. and mean cell volume was 40.0 ± 0.07 per cent.

It may be concluded that normal standards for hemoglobin concentration, red cell counts and cell volumes must be interpreted as a range of values which is wider than the ranges recorded by earlier studies.

REFERENCES

- (1) SACHS, A., V. E. LEVINE, F. C. HILL AND R. HUGHES. *Arch. Int. Med.* 71: 489, 1943.
- (2) SACHS, A., V. E. LEVINE AND W. O. GRIFFITH. *Arch. Int. Med.* 60: 982, 1937.
- (3) OSGOOD, E. E. *Arch. Int. Med.* 56: 849, 1935.
- (4) BARER, A. P., W. M. FOWLER AND C. W. BALDRIDGE. *Proc. Soc. Exper. Biol. and Med.* 32: 1458, 1935.
- (5) DUCKLES, D. AND C. A. ELVEHJEM. *J. Lab. and Clin. Med.* 22: 607, 1937.
- (6) SACHS, A., V. E. LEVINE AND A. APPELSIS. *Arch. Int. Med.* 52: 366, 1933.
- (7) WINTROBE, M. M. *Bull. Johns Hopkins Hosp.* 53: 118, 1933.
- (8) ANDRESEN, M. I. AND E. R. MUGRAGE. *Arch. Int. Med.* 58: 136, 1936.
- (9) CASTLE, W. B., G. R. MINOT AND H. A. CHRISTIAN. *Pathological physiology and clinical description of the anemias.* Oxford University Press, New York, 1936.
- (10) MAGATH, T. B., J. BERKSON AND M. HURN. *Am. J. Clin. Path.* 6: 568, 1936.
- (11) VAN ALLEN, C. M. *J. Lab. and Clin. Med.* 10: 1027, 1925.
- (12) LEICHSENRING, J. M., E. G. DONELSON, L. M. WALL AND M. A. OHLSON. *J. Lab. and Clin. Med.* 25: 35, 1939.
- (13) COHEN, B. AND A. H. SMITH. *J. Biol. Chem.* 39: 489, 1919.
- (14) TODD, J. E. AND A. H. SANFORD. *Clinical diagnosis by laboratory methods.* W. B. Saunders Company, Philadelphia, 1937.
- (15) PETERS, J. P. AND D. D. VAN SLYKE. *Quantitative clinical chemistry, 1st ed., Interpretations.* The Williams & Wilkins Co., Baltimore, 1932.

THE AFFINITY OF HEMOGLOBIN FOR OXYGEN AT SEA LEVEL AND AT HIGH ALTITUDES¹

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Adaptation to low atmospheric pressure is largely regulated by the length of exposure and atmospheric pressure. Individual differences are observed which are still not well understood. In consequence, in the study of a given response to a condition of anoxia it is essential to take into account both of these modifying factors before general conclusions are reached, some of which may appear to conflict with results obtained in previous and apparently similar investigations.

Among the various mechanisms of adaptation investigated those related to the quantity of circulating hemoglobin and its oxygen capacity have a considerable interest. There is general agreement that an increase in hemoglobin is a very constant response. There is less agreement, because fewer studies have been made, that the affinity of hemoglobin for oxygen changes when the body is exposed to an anoxic environment. The investigations here reported relate to the latter aspect, and it has been considered important to carry them out under similar experimental conditions in the different groups of subjects studied. The position of the oxygen dissociation curve has been determined in *a*, residents at sea level; *b*, Indian native residents at an altitude of 4,540 meters (14,890 ft.); *c*, some of the men included in group *a* were again studied within the first two hours after arrival at the higher altitude; and *d*, subjects included in group *b* were taken down to sea level and the same investigations were repeated also within the first two hours after arrival. Thus the results obtained may be related to a condition of anoxemia present since birth, as compared with the lack of it, and to the sudden development or suppression of such a factor.

PROCEDURES AND METHODS. Arterial blood was obtained from the radial artery in 17 healthy adult males, of an age between 21 and 28 years, residents of Lima, at sea level²; blood was again obtained, one to three weeks later, in 12 of these men within the first two hours after arrival at Morococha, in the Andean region, at an altitude of 4,540 meters (14,890 ft.), with an average barometric pressure of 453 mm. Hg. Blood from the same artery was also obtained in 12 Indian natives, 18 to 44 years old, residents of Morococha, at the above mentioned altitude, who were born in that locality or in nearby places; in 8 of these men, one to three weeks later, arterial blood was drawn

¹ Aided in part by a grant from The Rockefeller Foundation.

² Lima is located at an altitude of 150 meters (490 ft.); from the point of view of the investigations here reported this may be considered as sea level.

again within the first two hours after arrival at sea level. The average time of the ascent and descent, made by car, was three hours. Physical activity was restricted as much as possible. The resident subjects, at sea level and at high altitude, were in fasting condition; before ascent and descent a light breakfast (tea, without milk, and crackers) was usually allowed several hours before the blood sample was obtained. The blood, obtained anaerobically, was mixed with a solution of heparin (Connaught) and kept under mercury and in ice until analysis was carried out. Blood obtained at high altitude was transported to Lima. Time elapsed between puncture and equilibration and analysis varied between one and six hours. The oxygen dissociation curve was determined in each sample of blood (49 in total) following the techniques developed at the Fatigue Laboratory (Harvard University, Boston)³ (1, 2). The CO₂ and the O₂ content of the blood as drawn and of that equilibrated at pCO₂ = 40 mm. Hg and pO₂ of about 200 mm. Hg gave the degree of oxygen saturation and one point (T₄₀) on the CO₂ dissociation curve; pH was calculated by means of the Henderson-Hasselbalch formula. In residents at sea level and at high altitude five points on the oxygen dissociation curve were investigated, these corresponding to pO₂ of 15, 25, 35, 45 and 65 mm. Hg; the pCO₂ in the tonometers was set at 40 mm. Hg in the first group of subjects and at 32 to 36 mm. Hg in the second, this last pressure corresponding to their arterial pCO₂. In the repeated determinations made on blood taken after arrival at high altitude and at sea level, only four points on the curves were determined: at pO₂ of 25, 35, 45 and 55 or 65 mm. Hg; the pCO₂ in the tonometers was set to correspond to the arterial pCO₂ (from 34 to 38 mm. Hg). In men studied at high altitude and after arrival at sea level the arterial pCO₂ was determined previous to the equilibrations corresponding to the different points in the oxygen dissociation curve, in order to adjust the pCO₂ in the tonometers to that tension. All equilibrations were made at 37°C. The analysis of the blood samples was made in the Van Slyke manometric apparatus, using 0.5 cc. of blood, and of the gas equilibrating mixtures of the tonometers in the Haldane-Henderson apparatus.

The oxygen dissociation curves, at arterial pH and at pH 7.40, were plotted on logarithmic co-ordinates (log. pO₂ against log. 100Hb/HbO₂), and from the approximate straight lines, in which pO₂ for Hb = HbO₂ was located, the usual curves of per cent HbO₂ against pO₂ (mm. Hg) were calculated. The slope of the logarithmic curves, which is the constant "n" in the Hill-Barcroft equation, was calculated for the different groups of subjects studied. The line charts developed at the Fatigue Laboratory were used in the various calculations. The correction of the curves to pH 7.40 was made by means of the factor

$$\frac{\Delta \log pO_2}{\Delta pH} = -0.48$$

³ The authors wish to express their appreciation to Dr. D. B. Dill and associates of the Fatigue Laboratory (Harvard University) for the kind hospitality and facilities offered to them in various opportunities.

RESULTS. In table 1 are given the values of pO_2 (mm. Hg) for $Hb = HbO_2$ obtained in 17 men living in Lima, at sea level, and in 12 permanent residents of Morococha, at an altitude of 4,540 meters (14,890 ft.). These values tend to be higher, both at the arterial pH and at a standard pH 7.40, in the high altitude subjects. The calculated differences between the mean values found in the two groups: 2.66 ± 0.32 and 1.74 ± 0.36 mm. Hg, respectively, are statistically significant. This lower affinity of hemoglobin for oxygen at high altitudes, which is present at all points in the dissociation curve (fig. 1) is not related, apparently, to possible racial factors. The group of sea level residents included 10 subjects born at low places and 7 subjects who had been born in high altitude localities (between 2,200 and 3,800 m.) and of similar racial stock as those studied in Morococha, but who had been living in Lima for many years; the mean values of pO_2 corresponding to half saturation of the blood, and at pH 7.40, were almost identical in these two groups of men studied at sea level: 24.13 ± 0.11 and 24.77 ± 0.27 mm. Hg, respectively.

It is interesting to point out that the most marked shift to the right in the oxygen dissociation curve was observed in two brothers, residents at high altitude (listed as 11-Jama and 12-Aama in table 1), who showed very similar high pO_2 values for $Hb = HbO_2$.

Table 2 gives the position of the oxygen dissociation curve (at $Hb = HbO_2$) in 12 men studied within the first two hours after arrival to Morococha (altitude 4,540 m.). The values previously found at sea level, in the same subjects, are also given for comparative purposes. At arterial pH the affinity of hemoglobin for oxygen decreased slightly in 10 of the 12 subjects (83 per cent), and the difference between the mean pO_2 values found at sea level and at high altitude was 1.01 ± 0.24 mm. Hg. At a standard pH 7.40 the affinity decreased, also very slightly, in 9 of the men (75 per cent); in 1 it did not change and in 2 decreased. The mean difference between the sea level and the altitude values was found to be 0.63 ± 0.23 mm. Hg. No relation was observed between the changes developed on arrival at high altitude and the place of birth or race of the subjects studied.

In table 3 are given the values of pO_2 (mm. Hg) for $Hb = HbO_2$ in 8 men brought down from their permanent place of residence at high altitude (4,540 m.) to sea level, where they were investigated within the first two hours after arrival. The previous values obtained at high altitude are included for comparative purposes. Both at the arterial pH and at a standard pH of 7.40 no significant variations were observed in the position of the oxygen dissociation curve under the influence of a sea level pressure. The mean values, found under these different environmental conditions, were almost identical.

The mean values of the pressures of oxygen necessary for different degrees of saturation of the blood in the various groups of men studied are shown in table 4. The constant " n " of the Hill-Barcroft equation, which measures the slope of the logarithmic curve, has also been calculated; it may be noted that this constant differs moderately in the groups of residents but it does not appreciably change on arrival at high altitudes or at sea level.

TABLE 1

*Position of the oxygen dissociation curve in two groups of healthy adult men: a, residents at sea level; and b, Indian native residents at an altitude of 4,540 meters (14,890 ft.)**

(Arterial blood equilibrated at 37°C.)

N*	SUBJECTS	AT SEA LEVEL			
		HbO ₂ cap.	pH	pO ₂ for Hb = HbO ₂	
				At art. pH	At pH 7.40
		vol. %		mm. Hg	mm. Hg
1	Garc	23.01	7.40	23.99	23.99
2	Vent	22.61	7.42	24.77	25.41
3	Calo	22.86	7.39	24.55	24.38
4	Lede	21.94	7.39	24.27	24.10
5	Alda	21.76	7.37	24.55	23.71
6	Laqu	21.77	7.39	23.71	23.44
7	Brez	20.95	7.32	25.23	23.71
8	Riec	21.16	7.40	24.49	24.49
9	Gano	21.71	7.38	24.38	23.93
10	Hamm	21.44	7.46	23.60	24.15
11	Paz	22.58	7.36	26.42	25.35
12	Gonz	21.78	7.34	24.72	23.23
13	Matu	20.37	7.40	25.76	25.76
14	Tres	22.71	7.39	24.02	23.66
15	Llos	19.66	7.39	25.00	24.72
16	Care	22.13	7.41	25.94	26.12
17	Zuni	23.22	7.38	24.97	24.55
Mean \pm P.E.....		21.86 \pm 0.15	7.39 \pm 0.006	24.73 \pm 0.12	24.39 \pm 0.13
St. Dev. \pm P.E.....		0.93 \pm 0.11	0.04 \pm 0.004	0.75 \pm 0.08	0.81 \pm 0.09
C. of V. (%).....		4.3	0.5	3.0	3.3
		AT HIGH ALTITUDE			
		HbO ₂ cap.	pH	pO ₂ for Hb = HbO ₂	
				At art. pH	At pH 7.40
		vol. %		mm. Hg	mm. Hg
1	Gons	28.84	7.38	26.79	26.12
2	Cara	26.87	7.33	28.64	26.49
3	Mora	33.71	7.30	27.35	24.38
4	Barr	27.42	7.40	26.36	26.36
5	Ansi	27.15	7.40	25.76	25.76
6	Arti	29.52	7.35	26.30	24.89
7	Estr	29.24	7.38	28.05	27.42
8	Ordo	29.96	7.31	27.23	24.77
9	Quis	32.79	7.32	25.88	23.60
10	Espi	30.60	7.38	26.00	25.47
11	Jama	30.68	7.36	30.34	29.11
12	Aama	26.85	7.38	29.99	29.24
Mean \pm P.E.....		29.48 \pm 0.44	7.36 \pm 0.006	27.39 \pm 0.30	26.13 \pm 0.34
St. Dev. \pm P.E.....		2.16 \pm 0.31	0.03 \pm 0.001	1.50 \pm 0.22	1.68 \pm 0.24
C. of V. (%).....		7.3	0.4	5.5	6.4

* Average barometric pressure: 453 mm. Hg.

Previous investigations. The characteristics of the oxygen dissociation curve in healthy men living at sea level have been well established. Following the techniques developed at the Fatigue Laboratory (Harvard University), which are the ones used in the present study, several investigations (1-4), which include a total of 37 determinations made on healthy men, have shown that the pO_2 (mm. Hg) necessary to half saturate the blood hemoglobin varies within narrow limits. A statistical study of all these values, collected from the literature, and which correspond to a standard pHc of 7.10 and a temperature of 37-37.5°C, gives the following results: Mean \pm P.E. = 26.32 ± 0.14 mm. Hg;

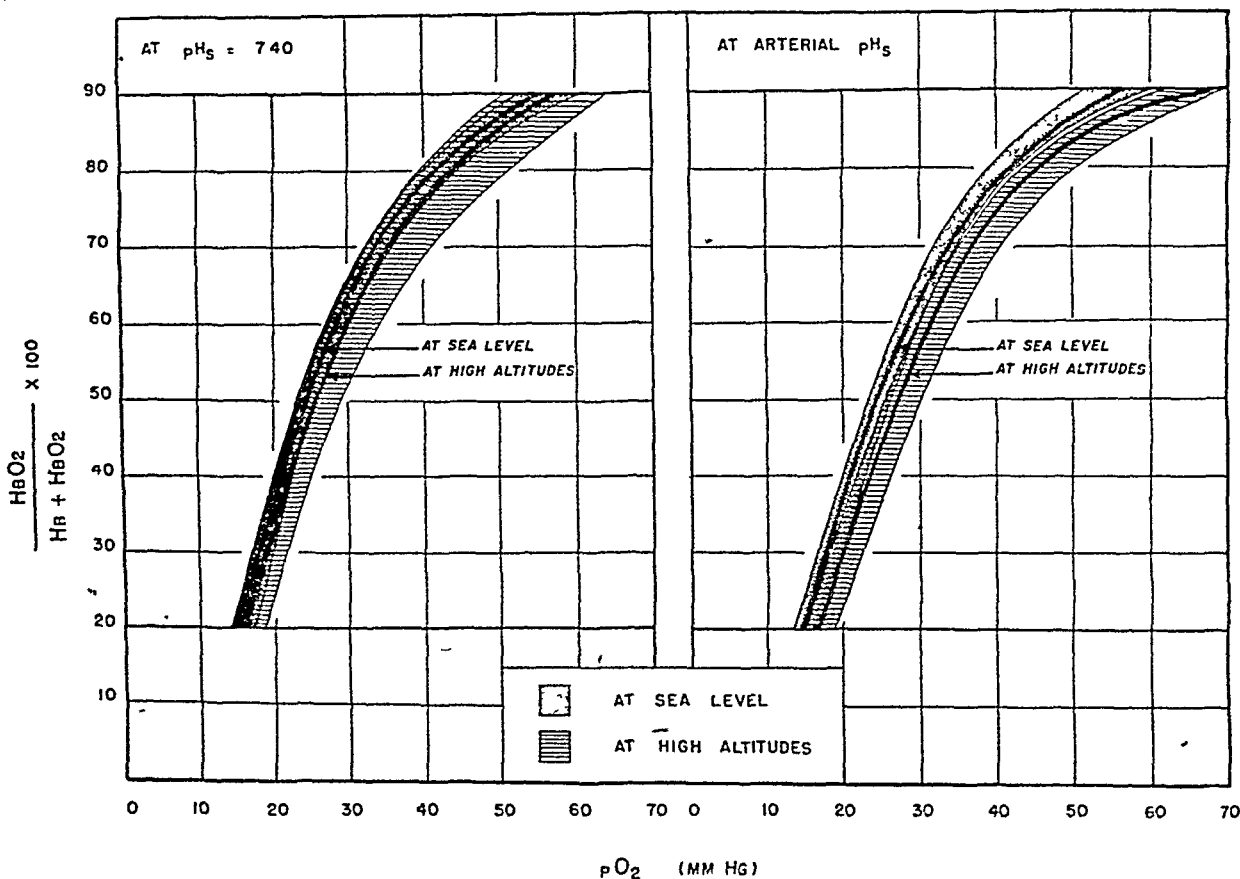


Fig. 1. Position of the oxygen dissociation curve, mean and range, in residents at sea level and at an altitude of 4,540 meters (14,890 ft.).

standard deviation \pm P.E. = 1.27 ± 0.10 mm. Hg; coefficient of variation = 4.8 per cent and extreme variations between 23.2 and 28.9 mm. Hg. Our mean of 24.39 ± 0.13 mm. Hg, obtained in the 17 men studied at sea level, differs by 1.93 ± 0.19 from that general mean value; the coefficient of variation observed in our results, which vary between 23.23 and 26.12 mm. Hg is slightly lower: 3.3 per cent. Darling and others (6) found in six healthy women, at a pH 7.40 and 37°C, a mean pO_2 of 26.03 mm. Hg for half saturation of the blood hemoglobin, this value being very similar to the one found in men.

The comparison of the "n" values obtained in our studies with those calcu-

lated from previous determinations made in normal subjects at sea level is given in table 4.

Less agreement is found in the results obtained at high altitudes in previous investigations. Douglas and others (7), in 1913, observed no change in the position of the oxygen dissociation curve of blood equilibrated with alveolar air after a few days and weeks of residence at an altitude of 4,300 meters (Pike's Peak). In 1922, from observations made in Cerro de Pasco (Peru), at an altitude of 4,330 meters, Barcroft and his collaborators (8) concluded that there

TABLE 2

Position of the oxygen dissociation curve in healthy adult men studied at sea level and within the first two hours after arrival to an altitude of 4,540 meters (14,890 ft.) (Arterial blood equilibrated at 37°C)

N°	SUBJECTS	PREVIOUS VALUES AT SEA LEVEL				ON ARRIVAL TO HIGH ALTITUDES			
		HbO ₂ cap.	pH	pO ₂ for Hb = HbO ₂		HbO ₂ cap.	pH	pO ₂ for Hb = HbO ₂	
				At art. pH	At pH 7.40			At art. pH	At pH 7.40
		vol. %		mm. Hg	mm. Hg	vol. %		mm. Hg	mm. Hg
1	Garc	23.01	7.40	23.99	23.99	24.60	7.37	25.35	24.49
2	Vent	22.61	7.42	24.77	25.41	20.54	7.36	26.85	25.53
3	Calo	22.86	7.39	24.55	24.38	22.53	7.40	23.33	23.33
4	Laqu	21.77	7.39	23.71	23.44	23.28	7.38	25.12	24.66
5	Ricc	21.16	7.40	24.49	24.49	22.22	7.33	26.49	24.49
6	Gano	21.71	7.38	24.38	23.93	23.20	7.41	24.89	25.23
7	Hamm	21.44	7.46	23.60	24.15	21.79	7.42	25.47	25.88
8	Matu	20.37	7.40	25.76	25.76	20.42	7.39	25.23	25.06
9	Tres	22.71	7.39	24.02	23.66	23.50	7.41	24.95	25.29
10	Llos	19.66	7.39	25.00	24.72	20.63	7.38	26.30	25.82
11	Care	22.13	7.41	25.94	26.12	22.99	7.40	26.55	26.55
12	Zuni	23.22	7.38	24.97	24.55	24.89	7.37	26.76	25.88
Mean ±P.E.....		21.89	7.40	24.60	24.55	22.55	7.39	25.61	25.18
St. Dev. ±P.E....		±0.21	±0.006	±0.14	±0.16	±0.29	±0.004	±0.19	±0.17
C. of V. (%).....		1.05	0.03	0.71	0.80	1.43	0.02	0.98	0.82
		±0.15	±0.004	±0.10	±0.11	±0.20	±0.003	±0.14	±0.12
		4.8	0.4	2.9	3.3	6.3	0.3	3.8	3.3

* Average barometric pressure: 453 mm. Hg.

was a shift to the left in the dissociation curve determined in the members of the expedition and in residents; the greater affinity of hemoglobin for oxygen was found to be more evident in the native Indians. This change, analogous to what has been later observed in fetal as compared with maternal hemoglobin in humans (9-12, 6) and in animals (13-17), was explained on the basis of an increased alkalinity of the blood in addition to a change in the affinity of hemoglobin for oxygen, independent of the pH.

Dill and others (3), in 1931, reported no appreciable change in the position of the oxygen dissociation curve after two to seven weeks of residence at an

TABLE 3

Position of the oxygen dissociation curve in healthy Indian natives studied in their place of residence: at an altitude of 4,540 meters (14,890 ft.) and within the first two hours after arrival to sea level*

(Arterial blood equilibrated at 37°C.)

N*	SUBJECTS	PREVIOUS VALUES AT HIGH ALTITUDES				ON ARRIVAL TO SEA LEVEL			
		HbO ₂ cap.	pH	pO ₂ for Hb = HbO ₂		HbO ₂ cap.	pH	pO ₂ for Hb = HbO ₂	
				At art. pH	At pH 7.40			At art. pH	At pH 7.40
		vol. %		mm. Hg	mm. Hg	vol. %		mm. Hg	mm. Hg
1	Cara	26.87	7.33	28.64	26.49	27.42	7.43	27.63	28.71
2	Mora	33.71	7.30	27.35	24.38	34.48	7.30	28.51	25.47
3	Arti	29.52	7.35	26.30	24.89	29.17	7.33	25.59	23.66
4	Ordo	29.96	7.31	27.23	24.77	30.12	7.33	25.59	23.77
5	Quis	32.79	7.32	25.88	23.60	32.54	7.33	26.18	24.21
6	Espi	30.69	7.38	26.00	25.47	29.08	7.36	27.16	25.94
7	Jama	30.68	7.36	30.34	29.11	29.72	7.34	29.65	27.61
8	Aama	26.85	7.38	29.99	29.24	25.29	7.37	27.67	26.67
Mean, ±P.E.		30.13	7.34	27.72	25.99	29.73	7.35	27.25	25.76
		±0.58	±0.01	±0.42	±0.51	±0.68	±0.01	±0.34	±0.44
St. Dev. ±P.E.		2.29	0.04	1.64	1.99	2.66	0.04	1.34	1.73
		±0.41	±0.007	±0.29	±0.35	±0.48	±0.007	±0.24	±0.31
C. of V. (%)		7.6	0.4	5.9	7.7	8.9	0.5	4.9	6.7

* Average barometric pressure: 453 mm. Hg.

TABLE 4

Oxygen dissociation curves in healthy men and women at sea level and at high altitudes
Comparative study between the mean values obtained in the various groups of subjects studied in the present investigation and those observed in previous studies

At pH 7.10 = pH 7.40—Blood equilibrated at 37–37.5°C.

% HbO ₂	PRESENT INVESTIGATIONS				PREVIOUS INVESTIGATIONS AT SEA LEVEL		
	Residents at sealevel	Residents at 4,540 m.	On arrival to 4,540 m.	On arrival to sea level	From Dill, Edwards and Conzolzazio (6) (In men)	From Keys and Snell (5) (In men)	From Darling and others (7) (In women)
	pO ₂ (mm. Hg)						
20	14.5	15.8	15.0	15.7	13.4		15.3
30	17.7	19.3	18.4	19.0	17.9		19.2
40	20.9	22.5	21.6	22.2	22.0	22.4	22.6
50	24.4	26.1	25.2	25.7	26.3	26.0	26.0
60	28.4	30.3	29.2	29.6	31.1	30.4	30.2
70	33.5	35.5	34.4	34.7	36.1	36.1	35.6
80	41.1	43.1	42.0	42.0	45.7	42.9	42.2
90	55.6	57.7	56.8	56.1	61.4		54.1
"n"*	2.66	2.77	2.69	2.81	2.50	2.73	2.65

* Slope of the logarithmic curves.

altitude of 3,280 meters; however, the analysis of their data shows that in 6 of the 8 members of the expedition there occurred some increase in the pO_2 (mm. Hg) corresponding to half saturation of the blood at pH 7.10 and $37.5^\circ C$; the mean value at sea level was 26.9 as compared with 27.8 mm. Hg at high altitudes. In 3 residents, at the above altitude, this value was found to be 28.7 mm. Hg, that is 1.8 mm. Hg higher than in residents at sea level. These figures give some evidence of a decreased affinity of hemoglobin for oxygen at high altitudes. In 1933, Buikov and Martinson (18) observed a left shift in the oxygen dissociation curve at altitudes between 600 and 2,100 meters. The most recent previous investigations have been made by the members of the expedition to the Andean regions of Chile; Keys, Hall and Guzmán Barrón (1), in 1936, observed a gradual shift to the right in the oxygen dissociation curve while increasing altitudes were reached, and after a period of residence which varied between few hours and several weeks in the different localities. At the altitudes of 3,660, 4,710, 5,340 and 6,140 meters, the mean pO_2 (mm. Hg) corresponding to $Hb = HbO_2$, at pH 7.10 and $37^\circ C$, became 1.6, 2.2, 2.9 and 2.8 mm. Hg, respectively, higher than at sea level. At 2,810 meters no change was found. In 3 residents at 3,660 meters the mean pO_2 was 1.7 mm. Hg higher than the sea level value obtained in the members of the expedition, but no difference was observed between this group and 8 residents at 5,340 meters. Hall (19), working with the same subjects, but using dilute solutions of hemoglobin, found no significant change at high altitudes, but observed a pO_2 of 26.0 mm. Hg at half saturation in the residents at 5,340 meters in contrast to 23.5 mm. Hg in the members of the expedition studied at the same altitude.

Native mammals and birds at high altitudes, investigated by Hall, Dill and Guzmán Barrón (20), showed, as compared with related animals living at sea level, a greater affinity of the blood hemoglobin for oxygen. Comparative observations were made at sea level and at high altitudes in the llama, an animal widely used for transportation by the Indians of the Andean regions; a left shift in the oxygen dissociation curve and a lower HbO_2 capacity were observed under conditions of low pressure. In sheep, in which the affinity of hemoglobin for oxygen is one of the lowest among mammals, no change was found at high altitudes.

DISCUSSION. The oxygen dissociation curve observed in our sea level subjects has the same general shape, but it is located slightly to the left of the one obtained by previous investigators in North American people. It is not possible to state, at the present time, whether this difference is due entirely to technical variations in the methods employed, or whether it is related, in part or wholly, to racial or other unknown environmental factors. These latter aspects, which have a particular interest in view of the possible existence of various forms of hemoglobin within the same species of animals or individual (21-23), have not yet been adequately investigated.

The results obtained in our investigations carried out at high altitude on newcomers and on residents, together with those found in previous studies made in recent years, furnish enough evidence to indicate that, at least in humans and

at altitudes over 4,000 meters, there is no change in the blood hemoglobin towards a greater affinity for oxygen and, in consequence, there is no similarity with the fetal type of hemoglobin as has been suggested. On the contrary, the evidence points to some decrease in the oxygen affinity of the blood hemoglobin. In the men studied immediately after arrival at high altitude the position of the oxygen dissociation curve, at a standard pH of 7.40, was practically identical to the one observed at sea level, but the fact that the large majority of them showed some degree of right deviation, under the low pressure environment, gives some significance to the slightly higher pO_2 (mm. Hg) necessary for half saturation of the blood at high altitude. In this respect our findings correlate with those previously obtained by Dill and others (3) and by Keys, Hall and Guzmán Barrón (1) after a considerably longer residence at high altitudes. The finding of a decreased affinity of blood hemoglobin for oxygen is somewhat more evident in the Indian native residents of high altitude, according to our investigations. The right deviation of the oxygen dissociation curve, together with its slightly more S-shape, as compared with sea level residents, is not entirely outside the possibilities of technical error, but it is significant that 10 of the 12 native residents studied at high altitude showed a pO_2 (mm. Hg) corresponding to $Hb = HbO_2$ higher than the mean sea level value. The difference was greater at the arterial pH, which was lower at high altitudes, but it remained statistically significant at a standard pH of 7.40 and $37^\circ C$. These findings agree with those observed by Dill and others (3) and by Keys, Hall and Guzmán Barrón (1), at lower altitudes (3,280 and 3,660 m., respectively) and by Hall (19) at 5,340 meters, the last investigator working with dilute solutions of hemoglobin.

The studies carried out in Leadville (3), at an altitude of 3,280 meters and in the Chilean Andes (1), at altitudes over 4,000 meters, seem to indicate that there are no changes in the electrolyte distribution between cells and plasma which would invalidate the comparison of results obtained at sea level and at high altitudes; in the latter studies, the direct determination of the pH by the glass electrode method gave results closely similar to the values calculated by means of the Henderson-Hasselbalch formula. The concentration of the hemoglobin in the circulating red cells is the same at sea level and at high altitudes (24, 25).

The slight decrease in the affinity of hemoglobin for oxygen, observed at high altitudes, may be interpreted as a favourable compensatory adjustment to the low pressure environment. It appears that under this condition the basic problem relates to the delivery of oxygen to the tissues, and a right deviation of the oxygen dissociation curve, even if slight, would be of appreciable benefit to this process, especially when we consider the increased quantity of blood hemoglobin present. It is interesting to call attention to the fact, which was originally demonstrated by Barcroft and his co-workers (8), and corroborated in later studies, that the arterial blood of men living at high altitudes leaves the lungs and carries to the tissues a higher amount of oxygen, as compared with men living at sea level, on account of the increased blood hemoglobin

which, in this respect, compensates for the lower saturation; yet this man has the limitations imposed by the high altitude environment due, largely, to the difficult delivery of oxygen to the tissues as a consequence of the low tension of this gas in the plasma. Our studies made in residents of a high altitude, immediately after they are brought down to sea level, show that there are no significant changes in the affinity of the blood hemoglobin for oxygen under this new environmental condition; the position and the shape of the oxygen dissociation curve remain virtually unchanged. This finding gives a greater significance to the changes which are observed when the reversal of the experiment is carried out.

SUMMARY AND CONCLUSIONS

The position of the oxygen dissociation curve has been determined in arterial blood obtained from *a*, 17 healthy adult men living at sea level; *b*, 12 Indian native residents of Morococha (Peru), at an altitude of 4,540 meters (14,890 ft.). The same determination has been repeated in 12 subjects of the first group within the first two hours after arrival at the high altitude, and in 8 men of the second group also within the first two hours after arrival to sea level. The results have been compared with those obtained in previous investigations.

There seems to be enough evidence to conclude that in humans, newcomers or residents, at altitudes of about 4,000 meters, or higher, there is no increased affinity of the blood hemoglobin for oxygen. On the contrary, the findings indicate a slight tendency toward a lower affinity, both at arterial pH and a standard pH of 7.40, especially in the native residents. This right shift in the oxygen dissociation curve at high altitude may be interpreted as a favourable compensatory adjustment to the low pressure environment.

REFERENCES

- (1) KEYS, A., F. G. HALL AND E. S. GUZMÁN BARRÓN. *This Journal* 115: 292, 1936.
- (2) DILL, D. B., A. GRAYBIEL, A. HURTADO AND A. C. TAQUINI. *Ztschr. f. Altersforsch.* 2: 20, 1940.
- (3) DILL, D. B., H. T. EDWARDS, A. FÖLLING, S. A. OBERG, A. M. PAPPENHEIMER AND J. H. TALBOTT. *J. Physiol.* 71: 47, 1931.
- (4) KEYS, A. AND A. M. SNELL. *J. Clin. Investigation* 17: 59, 1938.
- (5) DILL, D. B., H. T. EDWARDS AND W. V. CONSOLAZIO. *J. Biol. Chem.* 118: 635, 1937.
- (6) DARLING, R. C., C. A. SMITH, E. ASMUSSEN AND F. M. COHEN. *J. Clin. Investigation* 20: 739, 1941.
- (7) DOUGLAS, C. G., J. S. HALDANE, Y. HENDERSON AND E. C. SCHNEIDER. *Phil. Trans. Royal Soc. Ser. B.* 203: 185, 1913.
- (8) BARCROFT, J., C. A. BINGER, A. V. BOCK, J. H. DOGGART, H. S. FORBES, G. HARROP, J. C. MEAKINS AND A. C. REDFIELD. *Phil. Trans. Royal Soc. Ser. B.* 211: 351, 1922.
- (9) HASELHORST, G. AND K. STROMBERGER. *Ztschr. f. Geburtsch. v. Gynäk.* 100: 48, 1931.
- (10) EASTMAN, N. J., E. M. K. GEILING AND A. M. DE LAWDER. *Bull. Johns Hopkins Hosp.* 53: 246, 1933.
- (11) LEIBSON, R. H., I. I. LIKHNITZKY AND M. G. SAX. *J. Physiol.* 87: 97, 1936.
- (12) NOGUCHI, M. *Japan J. Obst. and Gynec.* 20: 358, 1937.
- (13) MCCARTHY, E. F. *J. Physiol.* 80: 206, 1933.

- (14) BARHOFF, J., K. H. E. ELLIOT, L. B. FLEXNER, F. G. HALL, W. HERKEL, E. F. MCCARTHY, T. MCCLURKIN AND M. TALAAT. *J. Physiol.* **83**: 192, 1934.
- (15) HALL, F. G. *J. Physiol.* **82**: 33, 1934.
- (16) HALL, F. G. *J. Physiol.* **83**: 222, 1934.
- (17) ROOS, J. AND C. ROMIJN. *J. Physiol.* **92**: 249, 1938.
- (18) BUIKOV, K. M. AND E. E. MARTINSON. *Arch. sci. biol. (USSR)* **33**: 147, 1933.
- (19) HALL, F. G. *J. Biol. Chem.* **115**: 485, 1936.
- (20) HALL, F. G., D. B. DILL AND E. S. GUZMÁN BARRÓN. *J. Cell. and Comp. Physiol.* **8**: 301, 1936.
- (21) GEIGER, A. *Proc. Royal. Soc., Ser. B* **107**: 368, 1931.
- (22) BRINKMAN, R., A. WILDSCHUT AND A. WITTERMANS. *J. Physiol.* **80**: 377, 1933.
- (23) BRINKMAN, R. AND J. H. P. JONXIS. *J. Physiol.* **85**: 117, 1935.
- (24) TALBOTT, J. H. *Fol. Hematol.* **55**: 11, 1936.
- (25) HURTADO, A., C. MERINO AND E. DELGADO. *Arch. Int. Med.* (to be published).

IMPROVED MEASUREMENT OF THE EFFECT OF INTRAVENOUSLY INJECTED ADRENALIN ON THE RESPIRATORY EXCHANGE BY COLORIMETRIC DETERMINATION OF CARBON DIOXIDE IN EXPIRED AIR AND CONTINUOUS GRAPHIC REGISTRATION OF OXYGEN CONSUMPTION

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Previous work in which the calorogenic action of intravenously injected adrenalin was determined from analysis of approximately 5-minute collections of expired air (Griffith et al., 1939, 1940) was felt at the time to be less than completely adequate in that it provided only featureless averages for intervals within which such a quickly-acting agent might be expected to produce transient effects of considerable peak-magnitude, especially during the period of injection when blood concentration is rapidly changing. In spite of inadequacy as to detail these earlier results seemed clearly to indicate that variations of oxygen intake and carbon dioxide output during and following intravenous administration of adrenalin were less an expression and measure of intrinsic modification of metabolic rate than of possible concomitant alterations in acid-base balance and the mechanics of pulmonary ventilation and the cardio-vascular system. Since at least the latter two are rapid in onset and subsequent mutability it became obvious that further analysis, at least of the immediate effect of adrenalin on respiratory exchange, demanded use of a method whereby oxygen intake and carbon dioxide output could be followed continuously and without interruption. Results with such a method are described in what follows.

APPARATUS. This was devised for use with anesthetized or otherwise immobilized animals to permit access to the hind legs for blood sampling or intravenous injection. It provides continuous, uninterrupted registration of oxygen intake and inspiratory minute-volume. Carbon dioxide concentration of the expired air (from which, and the volume of pulmonary ventilation, rate of output is calculated) is determined colorimetrically (Marriott, 1916; Higgins and Marriott, 1917; Osterhaut, 1918) at minute, or shorter intervals, adaptation of this to the present purpose having been greatly facilitated by the work of Winzler and Baumberger (1939) which appeared just as this was getting under way. Compared with other devices designed for somewhat similar use (Gesell and McGinty, 1926; Ledig and Lyman, 1927; Rein, 1933; Wollschitt et al., 1935; McAlister, 1937; Pfund and Gemmill, 1940) this is relatively inexpensive and simple in construction and operation.

Schematic diagrams with details of construction are provided by figures 1 and 2. As shown in figure 1, the anesthetized cat is placed so that the head, upper extremities, thorax and the abdomen to the hips are enclosed in the container, C. The tracheal cannula of the animal is connected through the wall of this

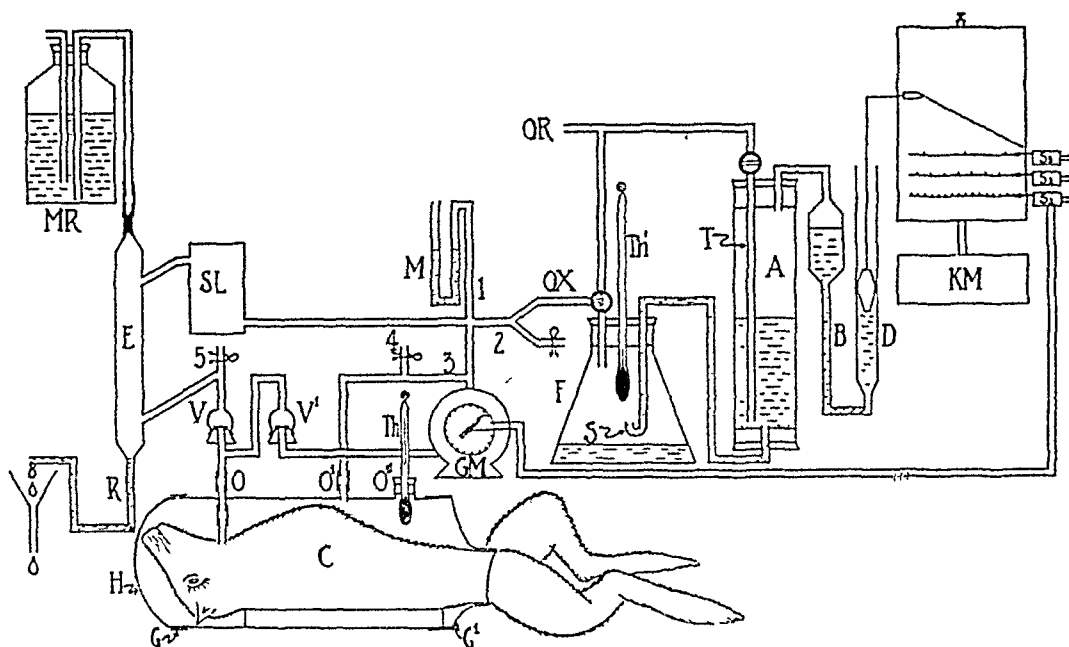


Fig. 1. The cat chamber, *C*, is a piece of metal tubing 4 inches in diameter and 10 inches long, intended to enclose all of the animal except the hind legs; these dimensions are for a 2 kgm. cat and could be altered to accommodate animals of other size. The two outlets, *O* and *O'*, are of $\frac{1}{2}$ -inch metal tubing; one of these, *O*, traverses the wall of the chamber about 2 inches from the head-end to connect the cat's tracheal cannula to the expiratory valve, *V*. A third outlet, *O''*, permits insertion of the thermometer, *Th*.

The headpiece, *H*, of the chamber is a copper water-bath basin, 4 inches in diameter, which accurately apposes this end of the metal tubing and is attached to it and the junction made air-tight by a 3-inch band cut from the wrist end of a surgeon's rubber glove, *G*.

Another surgeon's glove, *G'*, with fingers and thumb cut off, fits snugly over the shaved, lower abdominal portion of the cat's body just above the hips and is stretched to encircle this end of the chamber; when in place, this sleeve is so tightly stretched that it is not moved by the very slight variations of pressure within the chamber due to respiratory movements. Hence, with the cat's tracheal cannula connected to *O* by a short bit of rubber tubing and with the rubber sleeves over the headpiece and abdomen in place, the cat chamber becomes air-tight except for the connecting lead, *O'*, which allows communication between the enclosed volume of the chamber and the respiratory closed-circuit. Check against leaks before and after inclusion of the chamber in the circuit and again at the end of any experimental run is made with the water manometer, *M*.

The expiratory and inspiratory valves, *V* and *V'*, are constructed from thistle tubes, one-hole rubber stoppers, and rubber dental dam. The connection between the tracheal cannula and the T-junction between the valves is short enough so that its volume corresponds roughly with the respiratory dead space of the cat.

The equilibrator, *E*, is a vertically-placed glass tube 35 cm. long and of the same diameter (10 mm.) as the connecting tubing throughout the circuit. The inner surface is ground to a frosted appearance by coarse emery to facilitate uniform spread of the indicator solution which otherwise tends to descend in rivulets. Two homolateral side arms are attached about 30 cm. apart at an acute angle, so that pooling of indicator solution, as it flows down the inner surface of *E*, will not occur at the junctions. The upper end of *E* tapers rapidly into a section of 1 mm. capillary glass tubing; the lower end into thin-walled tubing of 4 mm. internal diameter. The capillary tubing at the upper end, which acts as inlet for the indicator solution, from the 15-liter Marriotte carboy, *MR*, is drawn out for a short to an internal diameter of a few-tenths of a millimeter so that considerable offered to the flow of solution. The thin-walled tubing of 4 mm. internal

container (at *O*) to inspiratory and expiratory valves, *V'* and *V*, which provide unidirectional flow of air driven around a closed circuit by the animal's own respiratory effort. From the expiratory valve, *V*, the expired air passes into an equilibrator, *E*, where it rises without obstruction against a downward-moving film of dilute methyl-red solution. The intensity of the acid (red) form of this solution, as it collects in the base of the equilibrator, in the reservoir, *R'*, is determined photometrically (fig. 2) and from it is calculated the concentration of CO_2 in the expired air. The expired air passes, next, to a can, *SL*, of soda-lime, wherein the CO_2 is completely absorbed. From this it then circulates past three side arms, 1, 2, 3, through a wet-test gasmeter, *GM*; which records the inspired volume, and through the inspiratory valve, *V'*, back to the cat by the force of its own inspiratory effort. The first side arm, 1, connects with a water manometer, *M*, which is used in testing for leaks; the second, 2, admits oxygen from a continuously-recording O_2 system as it is removed from the closed circuit by the animal; and the third, 3, connects with the cat chamber, thus preventing pressure fluctuations in the closed circuit that would otherwise arise from tidal respiration or change in the mid-position of the thorax.

Oxygen consumption might be recorded in any convenient way; that used here has been described by Schwabe and Griffith (1938) and repetition of detail is not necessary. In brief, the operation is as follows: as oxygen is depleted within the closed circuit described above it is replaced by entrance from *OX* through side arm, 2. This oxygen comes directly from the flask, *F*; the decrease of pressure thereby caused in *F* causes water to siphon over from the tube *A* and draws oxygen into *A* from a rubber basket-ball bladder (not shown) attached at *OR*. The lower end of the tube *T* in *A* is at the same level as the outlet of the siphon *S* in *F*, so that the movement of water from *A* to *F*, and, thereby, of oxygen through 2 into the closed respiratory circuit will occur at a pressure only very slightly less than atmospheric. The changing pressure (negative) above the water level in *A* is registered on smoked kymograph paper by the manometer and float, *BD*, and thus provides a continuous record of the rate of oxygen utilization by the animal. For further details, especially of re-setting for a new determination, reference should be made to the detailed instruction in the original description. For adaption to the present use, tube *A* is of such a size as will permit a 10- to 15-minute continuous run with average-size cats, without resetting; the

base serves as a collector, *R*, of the indicator solution and as the light-absorption chamber in the photocolorimetric set-up (see fig. 2).

The soda lime container, *SL*, is a copper can of 3-liters capacity which is filled with fresh soda lime at the start of each experiment.

A 1-liter, wet-test gasmeter, *Gm*, (American Meter Co.) has its large scale divided into 100, 10 cc. units. At each unit a wire contact is exposed so that, as a light spring attached to the pointer sweeps over the scale, the circuit of a signal magnet is closed once for each 10 cc. of air returning to the cat during inspiration. Thus the inspired volume is continuously registered on smoked paper to the nearest 10 cc. on the same kymographic record which records the oxygen consumption, as described in the text. The meter is carefully calibrated at a pulsing rate of flow equivalent to the cat's respiration (Krogh, 1920).

For other details see the text.

tubing of the siphon, *S*, is of large enough calibre to offer little resistance to the rapid flow of water through it; and a duplicate system is attached to the second arm of *Z*, so that the oxygen record need not be interrupted while either is being reset.

The photometer is shown diagrammatically and its construction described in the legend of figure 2; there is little about it that is critical and a simple optical system could be set up with other dimensions without sacrifice of its essential principles.

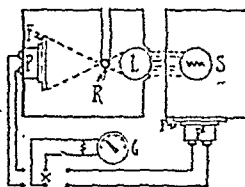


Fig. 2. The reservoir, *R*, attached to the base of the equilibrator (*E*, fig. 1) acts as the light-absorption chamber in this photocolorimetric arrangement.

A photocell exciter lamp, *S*, (General Electric, 8.5 V., 4.0 Amps., Bulb T-8, Fil. C-6), fed by two heavy storage batteries in parallel, provides a fairly constant light source. Greater constancy is obtained by immersing the bulb in a water bath containing dilute CuSO_4 , which serves the dual purpose of absorbing a large percentage of the infra-red rays given off by the light and preventing the formation of algae and molds.

Photosensitive cells, *P* and *P'*, of the photovoltaic or barrier layer type (General Electric; Cat. 88 x 565) are used to measure light intensities. One of these, *P*, measures the intensity of color of the indicator solution; the second, *P'*, placed an equal distance from the lamp, serves as a control, to measure possible changes in intensity of the light source.

Wratten Filters no. 74 (*F* and *F'*) limit the wavelength of transmitted light to a narrow band at about 530 mμ, the approximate wavelength of light absorbed by methyl-red in the red (acid) form. They cover the faces of the photocells completely.

Interposed between the light source and the indicator solution in *R* is a 20 mm. test tube, *L*, filled with dilute CuSO_4 solution, which acts as a cylindrical condensing lens to concentrate the light onto the reservoir tube, *R*, placed parallel to and immediately behind it. The light rays then diverge through a distance of $3\frac{1}{2}$ inches to cover the photocell, *P*.

Both photocells are connected to a L. and N. type R galvanometer, *G*, (sens. 0.5 microvolt, per. 5 sec., res. 12 ohms), through the opposite poles of a D.P.D.T.-mercury switch. Another mercury switch is placed so that the rest of the circuit may be excluded from the galvanometer and its critical damping resistance. The galvanometer deflection is read on an inverted meter-stick at a distance of three meters, hence the deflection is proportional to the current.

The indicator solution used is a weakly buffered solution of methyl-red. It is prepared from a stock solution containing 1.5 grams of indicator in 200 cc. of 95 per cent ethanol to which is added 300 cc. of 0.1 N NaOH; for use, 100 cc. of this stock solution is added to 15 liters of distilled water, resulting in a concentration of 0.02 gram of methyl red per liter.

This weakly-buffered, dilute methyl-red solution is siphoned on the Mariotte principle from a large 15-liter carboy (*MR*, fig. 1) into the inlet of the equilibrator at a rate which is determined by the height of the carboy above the equilibrator and the amount of resistance afforded by its constricted inlet. A rate of flow of 2.5 cc. per minute was found optimal for this work. The volume of solution in the

reservoir exposed to the incident light is about 1.2 cc., hence, at this rate of flow the reservoir-absorption chamber is completely refilled by a freshly equilibrated solution every 30 seconds. This assumes minimal mixing of solution in the reservoir, but this does not seem unfair inasmuch as the fresh solution is very slowly entering from wet sides.

This type of equilibrator was finally adopted after much trial with bubbling devices of various kinds because: throughout an experiment of several hours' duration it provided continuously an indicator solution of standard composition, unaffected by evaporation or contamination; secondly, it effected a more rapid equilibration than any other except sintered-glass bubblers, but without the resistance to air flow which rendered these impossible of application in an air stream moved by the animal's own expiratory effort; and finally, the equilibrated solution in the reservoir being undisturbed by bubbles, its color intensity can be determined continuously without interruption of the air circuit.

Whether or not the indicator solution which collects in *R* has reached equilibrium with the experimental gas will depend on the rate of replacement of the gas and also upon the length of the film of exposed indicator solution (30 cm.) and its thickness; this last will depend on the rate of flow and also, in actual use, upon certain practical details: the equilibrator must be vertical, otherwise rivulets which lessen the surface exposure are formed; also, roughening the inner surface of the equilibrator to a ground-glass consistency facilitates uniformity of spread of the solution; but even in spite of these precautions results were occasionally erratic until practice was made of cleaning the inner surface of the equilibrator with hot cleaning solution before each day's use; i.e., enough grease apparently accumulates on the surface with use to break up the otherwise smooth surface film.

In going suddenly from zero to 7 per cent carbon dioxide in the gas flowing through the equilibrator, equilibrium is 90 per cent complete in two minutes. Smaller differences in carbon dioxide content required less time for establishment of complete equilibrium, because the dilution effect is not so important and diffusion between the solution in the reservoir and that freshly entering it becomes less significant.

A linear relationship between CO_2 tension of the experimental gas and the ratio of incident to transmitted light has been described by Winzler and Baumberger as:

$$\left(\frac{I_0}{I} - 1\right) K = p\text{CO}_2 \dots \dots \dots (1)$$

(I_0) is the galvanometer deflection for the current generated in the photocell (*P*, fig. 2) by the light traversing the CO_2 -free (yellow) solution of methyl red.

(*I*) is the galvanometer deflection resulting from the current subsequently generated in the same photocell (*P*) by the light transmitted through the same solution after equilibration with a given tension of CO_2 .

(*K*) is a constant which includes the extinction coefficient, the quantity of light incident to the solution, the depth of the absorbing medium, etc. It is determined by equilibrating the indicator solution with a known concentration of CO_2

(in practice from a prepared tank of compressed gas containing approximately 95 per cent nitrogen and 5 per cent carbon dioxide). This is done before and after each experimental run, and values for (K) agreed on any given day within 3 per cent of each other, as calculated from the equation:

$$K = \frac{p\text{CO}_2}{\frac{I_o}{I} - 1} \dots\dots\dots (2)$$

If the light source could be kept constant, the CO_2 tension in the equilibrated gas could easily be calculated for any moment by substituting the galvanometer deflection for that moment (I) into equation (1) above, (I_o) and (K) having been previously determined. The light source, however, is never absolutely constant; hence, the second photocell (P') is used to measure directly the intensity of the light source, to permit correction of (I_o) by a proportionality factor. At the same time that the original galvanometer deflection (I_o) is obtained, a reading for the current generated (E) in the comparison photocell (P') is recorded also. Hence, any change in light source intensity is accompanied by a proportionate change in incident light, or

$$\frac{I'_o}{I} = \frac{E'}{E} \dots\dots\dots (3)$$

where (E) and (I_o) are as described above and (I_o) is the deflection which would result from the light traversing the CO_2 -free indicator solution at the same time that the altered intensity of the light source produces the galvanometer deflection for (P') of (E'). Or, the true incident light effect at any later reading subsequent to the original determination of (I_o) may be expressed as:

$$I'_o = I_o \times \frac{E'}{E} \dots\dots\dots (4)$$

This makes unnecessary the interruption of the experiment each time the intensity of the light source changes to introduce a fresh CO_2 -free solution for the purpose of obtaining a new (I_o).

Briefly, then, (I_o) is determined once and for all for the CO_2 -free indicator solution at the beginning of the run, as is also the comparative reading (E) on the control photocell. Whenever a subsequent reading (I') is taken, a corresponding reading (E') is taken with the control photocell. Then a known concentration of CO_2 is equilibrated with the indicator solution and (K) is determined; combining equations (2) and (4):

$$K = \frac{p\text{CO}_2}{\frac{I_o}{E} \times \frac{E'}{I} - 1} \dots\dots\dots (5)$$

The CO_2 concentration subsequently equilibrated with the indicator solution may be determined, combining equations (1) and (4):

$$p\text{CO}_2 = K \left(\frac{I_o}{E} \times \frac{E'}{I} - 1 \right) \dots\dots\dots (6)$$

In conclusion, then, we may summarize the resulting record. A smoked-paper kymograph record is obtained which shows: 1, the sloping oxygen curve; 2, a time signal; 3, a second signal-magnet record of the inspired volume (gasmeter); and 4, a third signal magnet record indicating the instants at which photometric readings were made. Each of the last signal-marks is numbered to correspond with the numbered record of the galvanometer deflections, including the deflection with each photocell and a zero determination. From these records it is a simple matter to compute the percentage of CO_2 in expired air, the inspired volume, and the volume of O_2 consumed for any given period; then:

$$\text{CO}_2 \text{ output per minute} = \frac{\text{Insp. min. vol.} - \text{O}_2 \text{ consumption}}{1 - \frac{\text{per cent CO}_2}{100}} \times \frac{\text{per cent CO}_2}{100}$$

Inasmuch as the record of inspired volume and O_2 consumption is continuous, and determinations of the percentage of CO_2 can be made as frequently as every 15 seconds, if desired, the method may be considered to be a continuous one of moderate accuracy for short periods. For one-minute periods CO_2 production may be calculated to within ± 3 per cent, the O_2 consumption to within ± 2 per cent of their respective volumes.

Checks were performed by substituting a rubber bulb for the animal's lungs, attaching it to the lower end of valve V in figure 1; rhythmical compression of the bulb by a motor-driven eccentric provided circulation of air in the closed system of the apparatus. To compensate for volume changes as the bulb was compressed and released, a rubber ice-bag, rigged up to act as a small bellows, was attached to the end of side-arm 3 in figure 1; this was inflated when the bulb was compressed and sucked empty when the bulb was released.

Alcohol checks. With this provision for artificial circulation of air in the system, an alcohol combustion chamber was inserted in the apparatus between the two valves, V and V' , of figure 1. By this means the complete reliability of the apparatus has been thoroughly established. In addition to the usual difficulties and precautions pertaining to successful operation of an alcohol check, one appeared for which we were totally unprepared; which, as a consequence, entailed months of fruitless effort before it was brought under control; and which may, therefore, be of interest to any who may attempt to apply alcohol checks in work of this type. There are apparently products of the incomplete combustion of alcohol which affect the color of methyl-red indicator. Successful checks were therefore obtained only when the alcohol was burned in contact with a minute chimney or mantle of platinum-iridium gauze which apparently catalysed the combustion nearly enough to completion so that the only factor significantly affecting the color of the indicator was the carbon dioxide being produced.

Routine checks. After the reliability of the apparatus in its final form was established by alcohol checks these were resorted to only occasionally and routine checking was done by admitting pure carbon dioxide and withdrawing air at determined and equal rates into and from the opposite arms of a mercury-filled U-tube. This consisted of a calibrated burette of 400 cc. capacity connected at

its lower end by heavy rubber tubing with a leveling-bulb of equal capacity. The upper end of this burette was connected by a 3-way junction with a supply of pure carbon dioxide and, also, to the closed circuit of the metabolism apparatus at 5, figure 1. Thus by lowering and raising the leveling-bulb the burette could be filled with carbon dioxide and this, in turn, discharged into the air current of the closed metabolism circuit, kept in circulation by the rhythmically compressed rubber bulb as described in the preceding paragraph. The upper, open end of the leveling bulb was provided with a rubber-tubing connection, which could be applied to side-arm 4, figure 1. Thus, with the burette full of carbon dioxide and the leveling-bulb raised, release of a screw clamp on the rubber tubing which connected them would permit mercury to run at controlled rates from the leveling-

TABLE 1

DATE	"INSPIRATORY" MINUTE-VOLUME	CARBON DIOXIDE			"OXYGEN CONSUMPTION" (AIR WITH- DRAWN PER MINUTE)	RESPIRATORY QUOTIENT: CO ₂ CALCULATED "O ₂ " MEASURED
		Per cent	Admitted (per minute)	Calculated (per minute)		
	cc.		cc.	cc.	cc.	
3/12/43	400	2.90	12.6	11.6	12.3	0.95
3/12/43	390	2.60	10.3	10.1	9.6	1.06
3/12/43	400	2.68	10.6	10.6	10.6	1.00
3/12/43	400	2.58	10.2	9.9	10.0	0.99
3/19/43	430	3.23	16.4	14.4	13.4	1.07
3/19/43	435	3.12	14.6	14.0	14.4	0.98
3/19/43	417	3.79	16.5	16.4	16.8	0.98
3/22/43	400	2.35	11.0	9.9	10.1	1.02
3/22/43	400	2.53	10.7	10.6	10.8	0.98
3/22/43	404	2.71	11.2	11.5	11.3	1.02
3/22/43	408	2.54	10.4	10.8	10.4	1.04
3/25/43	383	3.52	14.5	12.7	13.4	0.95
	393	3.18	12.0	11.7	11.6	1.01
Average.....				11.9*	11.9*	1.00

* These are approximately the average rates of oxygen consumption and carbon dioxide production of 2 kgm. cats; see Griffith et al., 1941.

bulb into the burette; this would force pure carbon dioxide from the burette into the apparatus at 5 and withdraw an equal volume of air into the leveling-bulb above its mercury from the closed circuit at 4. The theoretical "respiratory quotient" of such a procedure should be 1.00; table 1 records values actually obtained in the course of one of the latest experimental periods extending over approximately two weeks.

EXPERIMENTAL. Procedure. The experiments were done on male and non-gravid female cats, ± 2 kgm. in weight, fasted 24 hours, or 4 to 6 hours after eating; neither sex nor alimentary status had noticeable effect on the results.

The anesthetic of choice would have been chloralose in order to maintain the conditions of these experiments as nearly as possible like those done previously; but, since this could not be obtained at the time, on account of the war, recourse

was had to urethane in full dose, or in half this amount, supplemented by half the recommended dose of nembutal (Pentobarbital Sodium, Abbott). This combination was used to secure a narcosis less depressant than that resulting from urethane in full strength, but more prolonged than produced by nembutal, alone. Both anesthetics were administered by subcutaneous injection.

Following induction of anesthesia the lower part of the abdomen just above the hips was clipped and shaved all around in order to provide a skin-smooth, airtight contact for the rubber cuff which sealed this end of the cat into the chamber of the metabolism apparatus, which, as described above, enclosed all of the animal except the projecting hind legs. Insertion was then made of a tracheal cannula and of a cannula for injection into a superficial branch of the femoral vein in the middle of one thigh.

The animal was then placed in the chamber of the respirometer and left undisturbed for 30 to 45 minutes to permit stabilization, especially of animal (rectal) and chamber temperatures. To this end the chamber of the apparatus rested on an electrically heated animal-holder and was covered, and the projecting hind legs wrapped, with insulating cotton batting. A small study lamp placed above and close to the chamber provided an auxiliary, gentler means of heat control. By these means there was no difficulty in maintaining the temperature of both chamber and animal constant for the half-hour experimental periods. Animal temperatures were kept within the relatively normal range of 38 to 40°C.

At the conclusion of this preliminary period of acclimation, determination of the normal respiratory metabolism was begun. While this was in progress, preparation was made for injection of adrenalin via the cannulated, superficial branch of the femoral vein in the thigh. Injection was begun as soon as sufficient record of the normal respiratory exchange was obtained and without the record being stopped or its continuity interrupted. Injection was always continued for exactly 5 minutes. Then, again, without stop or interruption of continuity, the record of the respiratory exchange was continued another 20 to 25 minutes. A rest period of 30 minutes intervened before beginning another experimental run, including establishment of a new "normal," the 5 minute injection and the 20-25 minutes succeeding. As a rule each animal received injections at each of the five rates mentioned below and usually in the order there given, beginning with the least and ending with the greatest.

The preparation of adrenalin used was the 1:1,000 Parke-Davis solution of the chloride. This was diluted with isotonic NaCl to produce concentrations which when injected at the rate of 1 cc. per minute (by hand from a 5 cc. syringe) provided rates of administration of 0.00025, 0.00050, 0.00100, 0.00200 and 0.00400 mgm. per kilo of body weight per minute.

Qualitative confirmation of the results to be reported was obtained in a number of experiments which were discarded for final quantitative consideration because of minor technical faults. The final conclusions are based on averages of 8 or 9 determinations of the effect of each rate of injection of adrenalin in as many different animals.

RESULTS. Figure 3 presents the grand averages of all (42) experiments and will

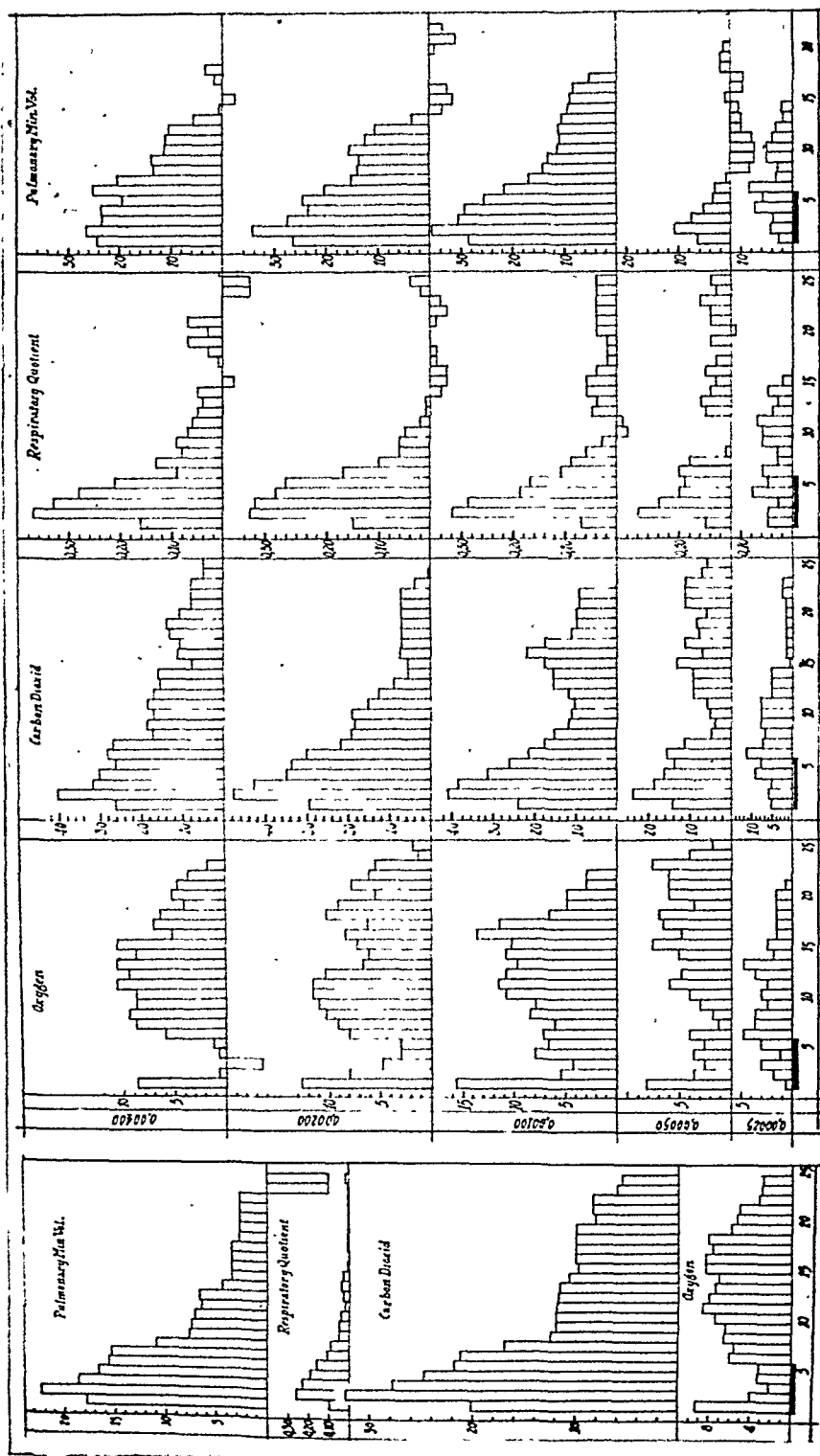


Fig. 3

Fig. 3. Grand averages of all (42) injections showing the effect of adrenaline on (from bottom to top) oxygen intake, carbon dioxide output, respiratory quotient and pulmonary minute volume during and following intravenous injection at rates from 0.00025 to 0.00400 mgm. per kilo of body weight for 5 minutes. Abscissae, time in minutes from beginning of injection (heavy line). Ordinates, per cent change in (from left to right) oxygen intake, carbon dioxide output, respiratory quotient and pulmonary minute volume caused by 5-minute, intravenous injection of adrenaline at rates (from bottom to top) of 0.00025, 0.00100, 0.00200 and 0.00400 mgm. per kilo body weight per minute. Abscissae and ordinates as in figure 3.

Fig. 4

be useful as establishing the average effect produced by intravenously injected adrenalin at the rates of administration used in this work (0.00025–0.004 mgm. per kgm. per min.). Within this range, however, the effect varies with the dosage; figure 4 indicates qualitatively the nature of this variation and, in spite of minor irregularities due to the relatively few determinations (8 or 9) with each rate of injection probably provides a fairly quantitative appraisal of it.

Whether the calorigenic action of adrenalin is due to direct cellular stimulation or oxidative removal of excessively liberated lactic acid, the course of the response might be expected to follow that shown in figure 4 following injection at the lowest rate of 0.00025 mgm. per kgm. per min. Oxygen intake, carbon dioxide output and pulmonary ventilation all increase gradually as injection proceeds to maximum of +4.8, +11.1 and +8.4 per cent, respectively, at its termination and then gradually return to normal. At all the higher rates of injection this type of response, with the maximum delayed into the interval 5 to 10 minutes after injection and the total action prolonged is also apparent with oxygen intake; carbon dioxide output still retains some evidence of it following injection at 0.0005 and 0.001 mgm. per kgm. per min.; but pulmonary ventilation following all but the lowest rate of injection, and carbon dioxide output following the two highest rates are completely dominated by an initial augmentation which is completely lacking in the supposedly fundamental calorigenic response to the minimally effective rate of administration. With oxygen intake, also, an initial abrupt and transient increase is added to the basic pattern at all of the four higher rates of injection.

As for pulmonary ventilation, following the four higher rates of administration, its behavior is perhaps easily explained on the basis of two well-known effects of intravenously injected adrenalin: the lactacidemia would provide an immediate stimulation probably proportional, within this range, to adrenalin dosage; the hypertension, acting via the inhibitory aortic and carotid sinus reflexes, would tend to counteract this stimulating influence, also in rough proportionality with dosage. The results would seem to indicate that these integrate to permit maximal augmentation of approximately 36 per cent occurring during the 2nd minute of injection following rates of administration of 0.001 and 0.002 mgm. per kgm. per min.; at the highest rate of administration the inhibitory influence would appear to gain over stimulation so that the increase is reduced. And, finally, this non-metabolic control of pulmonary ventilation seems completely to mask and obscure any alteration in it that might be attributable to a specific metabolic effect.

In this light, the behavior of carbon dioxide output following the higher rates of administration appears easily explained. Auspumpung, roughly proportional to the extraneously augmented pulmonary ventilation would explain the sudden, abrupt increase with its maximum during the 2nd minute of injection to a value of approximately +48 per cent following injection at the rate of 0.002 mgm. per kgm. per min.; and the reduction of this augmentation, comparable with the lesser increase of pulmonary ventilation, following the highest rate of adminis-

tration. Nor is it surprising that a compensatory retention might distort or completely obscure a delayed, intrinsic metabolic effect such as is suggested in the results with the three lowest rates of injection.

The above analysis bears heavily on previously published results (Griffith et al., 1939, 1940), but adding much in refinement of detail which was impossible of detection with the less delicate method of measurement then used.

Oxygen intake presents another and entirely new problem in the immediate, abrupt and transient increase which these results show to occur maximally during the first minute of injection at all rates above the minimal. Nothing in our own previous experience nor known to us in the literature prepared for it. Attempted explanation must therefore be cautiously tentative and subject to revision in the light of further work. One well established effect of adrenalin might, however, contribute to such a result. Contraction of blood depots, and especially the spleen and liver, resulting in the well-known adrenalin polycythemia, would bring into the circulation anoxic red cells whose oxygenation on first passage through the lungs might entail such an immediate, abrupt increase of oxygen intake. If proportional to dosage the comparative effects of 0.0005 and 0.001 mgm. per kgm. per min. would be explained. The progressive decrease of this effect with the two highest rates of injection demands, however, the countervailing action of some additional effect of adrenalin which begins to be dominant at the higher rates of administration. Here the greatest caution is necessary; it might not be impossible, however, that constriction, or congestion in the pulmonary circuit, becoming prominent with the higher rates of administration and for which there is some, but not conclusive evidence, might reduce the rate of oxygenation and, even, as with 0.004 mgm. per kgm. per min., momentarily to below normal. In any event, once oxygenation of these additional red cells was complete, oxygen intake would abruptly decline to the gradually ascending level of the calorigenically augmented oxygen utilization; this, thereafter, would establish the characteristics of the curve without the distortion due to auspumping and compensatory retention which the extrinsically controlled pulmonary ventilation impresses on the curves of carbon dioxide output.

Increased oxygen consumption by the stimulated heart is undoubtedly a part of the calorogenic action of adrenalin and might be supposed to show its effect immediately and abruptly; but, though contributing from the start to the increased intake, it is improbable that, by itself, it could be responsible for much of the large initial augmentation seen here. Also, occasional records secured by us indicated that within the range of dosages used in this work the most characteristic response of the heart was a gradual increase in rate during the first three or four minutes of injection; consequently it could play no part in the abrupt decline which sets in after the maximum increase of the first minute.

If this analysis possesses any element of truth, it is obvious respiratory quotient is altogether without metabolic significance as measured in these experiments; naturally and obviously it is dominated by the gross distortion of carbon dioxide output and except as a matter of record deserves no further consideration.

SUMMARY

A method is described for continuous, uninterrupted determination of the respiratory exchange of anesthetized or otherwise immobilized animals under experimental conditions permitting intravenous injection, blood sampling or other manipulation of the hind extremities.

All of the animal except the hind legs is enclosed plethysmographically in a container which is part of a closed system. The oxygen required to replace that used by the animal is recorded graphically and continuously and with an accuracy of ± 2 per cent. The carbon dioxide of the expired air is equilibrated with methyl-red solution, the intensity of color being determined photo-electrically; from this and the volume of inspired air, as recorded continuously by a gas meter in the closed circuit, carbon dioxide output can be computed with an accuracy of ± 3 per cent for intervals as short as one minute.

By this means continuous record has been obtained of the effect on respiratory metabolism of adrenalin injected intravenously for 5-minute periods at rates of 0.00025, 0.00050, 0.00100, 0.00200 and 0.00400 mgm. per kgm. per min. into cats under urethane-pentobarbital anesthesia.

The lowest and minimally effective dose has a simple, straightforward calorogenic action in which oxygen intake, carbon dioxide output, pulmonary ventilation and respiratory quotient increase slowly during the injection to a maximum at its termination and followed by gradual return to normal. This response, increased and prolonged, is apparent, especially in the oxygen intake, also with the higher rates of administration; but, at these higher rates it is obscured at the beginning by the appearance of other types of action of entirely different character.

For oxygen intake this initial response at the higher dosages consists of an immediate, sudden increase which is maximal within the first minute of injection and then almost as rapidly subsides in spite of continuing administration at uniform rate. During the 3rd-4th minute of injection the declining limb of this initial increase meets with and is reversed by the gradual rise associated with the slowly augmented phase of adrenalin action so that the curve comes to have a bimodal appearance.

Carbon dioxide output at all rates of administration above the minimal also shows the new appearance of an immediate, sudden outburst. This differs in type however from the coincident increase of oxygen intake; the maximum occurs during the second rather than the first minute of injection; and the subsidence toward normal is more gradual and prolonged. Because of this last and probably also due to retention to compensate for this initial, massive "auspumpung" the delayed rise which is so evident as a second hump on the oxygen curve is less evident or entirely obscured with carbon dioxide output.

It is suggested that the initial sharp rise in oxygen intake, when it occurs, may be associated with oxygenation of anoxic red cells expressed from blood depots by the higher concentrations of adrenalin. This should be sudden and transient and once complete would permit intake again to follow utilization as a measure of calorogenic action.

The initial outburst of carbon dioxide is probably due to displacement of it from the blood by lactic acid acidosis and massive elimination by the stimulated pulmonary ventilation.

Under these conditions, respiratory quotient must be altogether without metabolic significance.

REFERENCES

- (1) GESELL, R. AND D. A. MCGINTY. *This Journal* 79: 72, 1926.
- (2) GRIFFITH, F. R., JR., F. E. EMERY AND J. E. LOCKWOOD. *This Journal* 128: 281, 1940; 129: 155, 1940; 130: 197, 1940; 131: 561, 1941.
- (3) GRIFFITH, F. R. JR., J. E. LOCKWOOD AND F. E. EMERY. *This Journal* 126: 299, 1939; 127: 415, 1939.
- (4) HIGGINS, H. AND W. MCK. MARRIOTT. *J. Am. Chem. Soc.* 39: 68, 1917.
- (5) KROGH, A. *Biochem. J.* 14: 282, 1920.
- (6) LEDIG, P. G. AND R. S. LYMAN. *J. Clin. Investigation* 4: 495, 1927.
- (7) MARRIOTT, W. MCK. *J. A. M. A.* 66: 1594, 1916.
- (8) McALISTER, E. D. *Smithsonian Misc. Collect.* 95: #24, 1, 1937.
- (9) OSTERHOUT, W. J. V. *J. Gen. Physiol.* 1: 17, 1919.
- (10) PFUND, A. H. AND C. L. GEMMILL. *Johns Hopkins Hosp. Bull.* 67: 61, 1940.
- (11) REIN, H. *Arch. f. exp. Path. u. Pharmacol.* 171: 361, 1933.
- (12) SCHWABE, E. L. AND F. R. GRIFFITH, JR. *J. Nutrition* 15: 187, 1938.
- (13) WINZLER, R. J. AND J. P. BAUMBERGER. *Ind. and Eng. Chem.* 11: 371, 1939.
- (14) WOLLSCHITT, H., W. BOTHE, H. RUSKA AND E. G. SCHENCK. *Arch. f. exp. Path. u. Pharmacol.* 177: 635, 1935.
- (15) WOLLSCHITT, H. AND G. KRAMER. *Arch. f. exper. Path. u. Pharmacol.* 178: 378, 1935.

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